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CONTENTS OF VOL. 30, No. 2

PAGE

1. War problems and post-war responsibilities of the Association. By HUBERT MARTIN. (With Frontispiece) 109
2. Nitrogen nutrition of the onion. By R. M. WOODMAN 116
3. Behaviour of the fungus *Rhizoctonia solani* Kühn in the soil. By I. D. BLAIR, M.AGR.SC. (N.Z.), PH.D. (LOND.) 118
4. Experiments to determine the effect of certain wood preservatives on the growth and cropping of the cultivated mushroom (*Psalliota campestris*). By N. H. PIZER and H. H. GLASSCOCK . . 128
5. Value of phloem necrosis in the diagnosis of potato leaf-roll. By F. M. L. SHEFFIELD, D.Sc. (With Plate 2) 131
6. The moisture content of timber in relation to attack by *Lyctus* powder-post beetles. By E. A. PARKIN, M.Sc., PH.D., D.I.C. (With 3 Text-figures) 136
7. Experiments on the poisoning of honeybees by insecticidal and fungicidal sprays used in orchards. By C. G. BUTLER, D. J. FINNEY and P. SCHIELE. (With 2 Text-figures) 143
8. Field studies of potato-root eelworm, *Heterodera rostochiensis* Wollenweber, 1938–40. By H. W. MILES, D.Sc., PH.D., V. E. HENDERSON, B.A. and M. MILES, M.Sc. 151
9. Observations on the biology of the potato-root eelworm, *Heterodera schachtii* Schmidt. By A. E. W. BOYD 157
10. Stimulation of larval emergence in *Heterodera schachtii* Schmidt, by certain concentrations of silver compounds. By A. E. W. BOYD 161
11. Field experiments on the control of sheep maggots. By W. ROGIE ANGUS, I. THOMAS and O. G. WILLIAMS. (With 3 Text-figures) 164
12. Studies in the mechanism of fungicidal action. V. Non-metallic and sodium dithiocarbamic acid derivatives. By A. F. PARKER-RHODES. (With 4 Text-figures) 170
13. Preliminary experiments on the use of oil-soluble copper compounds as fungicides. By C. J. HICKMAN, R. W. MARSH and E. H. WILKINSON 179
14. Experiments on the rate of spread of narcissus stripe in the field. By LILIAN E. HAWKER . . 184
15. On the control of potato skin spot disease. By C. E. FOISTER 186
16. Note: Virus nomenclature. By G. C. AINSWORTH 187
17. Proceedings of the Association of Applied Biologists: 4 December 1942. Symposium on the honey-bee:
    - The position of the honey-bee in the national economy. Adult bee diseases. By C. G. BUTLER 189
    - Brood diseases of the honey-bee. By P. S. MILNE 191
    - Orchard spray poisoning of the honey-bee. By G. A. CARTER 195
    - Work on bee repellents. Management of colonies for pollination. By C. G. BUTLER . . 195
    - The design and interpretation of bee experiments. By D. J. FINNEY 197
    - The discussion. Compiled by E. P. JEFFREE 197
18. Report of the Council of the Association of Applied Biologists for the year 1942 198
19. Report of the Honorary Treasurer for the year ended 31 December 1942 199

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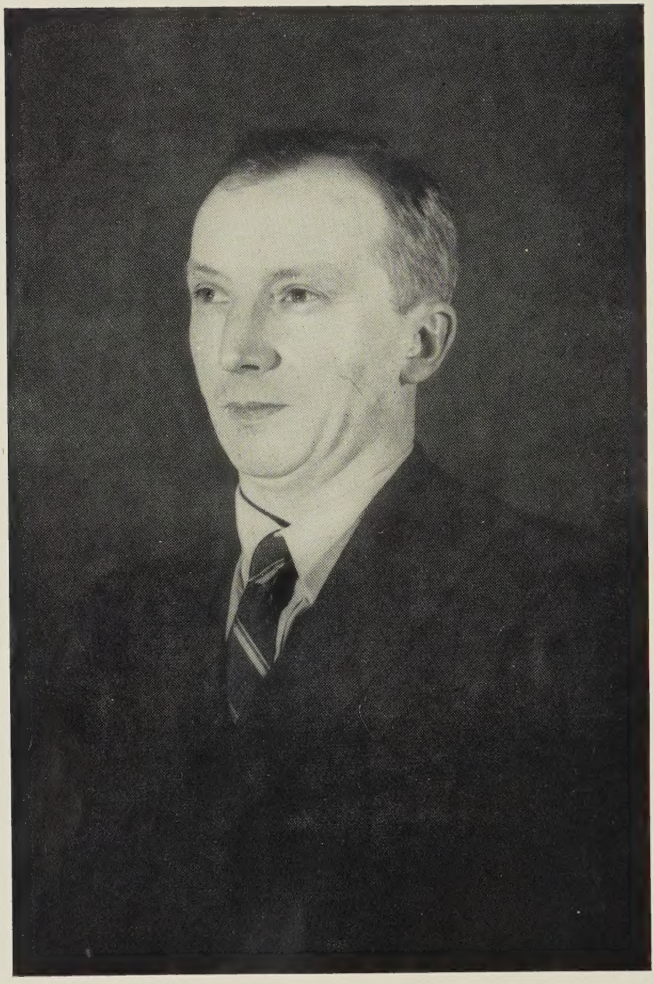

DR HUBERT MARTIN

President of the Association of Applied Biologists, 1941–1942

War problems and post-war responsibilities of the Association

BY HUBERT MARTIN

Agricultural and Horticultural Research Station, Long Ashton, Bristol

Address of the President of the Association of Applied Biologists delivered to the Annual General Meeting on Friday, 12 February 1943

(With Frontispiece)

Foremost among the strategic lessons of the last war was the vital importance of our agriculture, a lesson which seemed so quickly forgotten in peace yet which, to the credit of those responsible, was at once applied when war loomed anew. The immediate adoption of the policy of maximum food production fixed the war task of those members of our Association who have to do with crops and livestock, in particular, the Research and Advisory Services. Unfortunately, their prompt recognition of the part they were to play was frustrated by the procedure adopted in implementing the food-production campaign. At the outbreak of war, plans formulated under the Emergency Powers (Defence) Act of 1939 were put into operation and the direction and supervision of the campaign was delegated to the County War Agricultural Executive Committees. For some months, it seemed that the Advisory Services had been forgotten and that biology was to be denied the opportunity of contributing, in full measure, to the war effort.

When, two years ago, it was my privilege to accept the honour of election as your President, a feeling of frustration was common to all members of the Association and had found expression in the discussion on 'The Function of Applied Biology in Wartime' at our meeting of 6 Dec. 1940. At that meeting the Council was instructed to bring to the notice of the proper authorities the existence of problems of applied biology and to discuss with them the means of utilizing to the best advantage the services of applied biologists.

THE BIOLOGY WAR COMMITTEE

The misgivings which prompted this instruction were shared by members of the British Ecological Society and of the Society for Experimental Biology, and these societies joined our Association in the approach to the appropriate official bodies, namely, the Agricultural Research Council, Medical Research Council, and Department of Scientific and Industrial Research. The outcome of this movement was the appointment of the Biology War Committee, representative of all branches of biology and given terms of reference enabling it to advise the official organizations on all biological matters pertaining to the war effort. Further, it was to act as a channel for the two-way flow of ideas and problems to and from individual biologists and the official services. The Committee thus established a chain between those biologists with no direct contact with official research organizations and the authorities responsible for the direction of the national effort.

During my service on this Committee I have been encouraged by the number and variety of the problems and suggestions which have come before it. A score or more items, ranging from surveys of our natural resources and their fuller utilization to replace or to supplement imported material to *ad hoc* matters arising from war conditions, have been the subject of recommendations to the official organizations. When it is remembered that the Committee is not concerned with the projects of those biologists attached to centres under the supervision of the Research Councils, the report of the Committee's work, when published, will reveal that the suspicions which prompted the formation of the Committee were not unwarranted.

THE ADVISORY SERVICES

I have mentioned that the Biology War Committee is not directly concerned with biologists in established connexion with the official research organizations. How then have the Advisory and Research Services fared since their boycott in the transference of the implementation of agricultural policy to the County War Agricultural Executive Committees? Though still unmentioned in the *Notes on Agricultural Policy* issued by the Ministry of Agriculture in the spring of 1942, a recognition of their potential value was not long deferred and, in most counties, their recruitment by the Executive Committees has somewhat remedied matters. So far as I can judge the Provincial Advisers in the biological subjects may soon be expected to exert their proper and powerful influence on the activities of the War Agricultural Executive Committees and, in spite of their early war difficulties, will take with both hands their chances of enhancing the great worth of the Advisory Services to agriculture and the community.

The official recognition of proprietary pest control products

Our Association has, whenever possible, directed attention to possible improvements in the status and working of the Advisory Services, for these services provide those personal contacts which are the surest route by which applied biology reaches agricultural practice. For example, war has brought added importance to fungicides and insecticides as methods of disease and pest control and has recruited amateurs and novices to the 'Growmore' campaign. The problem of the proprietary product was thus brought to a head, for among such products are to be found insecticides and fungicides, which being superior to the older home-made remedies in efficiency and foolproof character, are more suitable for official recommendation, especially to inexperienced users. The pre-war policy had been to defer official recognition until an agreed specification had been reached, which, fundamentally, is the only sound method, for it requires an adequate knowledge of the factors responsible for field efficiency. But the elucidation of these factors is usually a problem of long-term research, and an alternative device must be found if the benefits conferred by the reliable proprietary product are to be fully used in the 'Growmore' campaign.

I had previously brought this subject before the Association at their meeting on 15 March 1940. In the following month the British Mycological Society appointed a subcommittee to examine and to report on the then existing system of recommending the use of proprietary fungicides. In July of that year the British Mycological Society invited the co-operation of our Association, and a joint subcommittee was set up to examine the entomological and other aspects of the problem. From informal discussion it was learnt that any scheme involving legislation was unlikely to receive prompt official adoption, and that no scheme was likely to meet official favour unless the main difficulties which had frustrated an earlier recognition of proprietary products could be overcome. These difficulties were associated with two requirements: first, the inclusion of products the composition of which was known only to the maker; secondly, machinery for the extension of recognition to new products. To meet the first requirement, the subcommittee adopted the device of coding to prevent an unwitting disclosure of information which the maker of a 'secret' product feared might help a competitor. The second difficulty required the establishment of a testing organization, and particulars of methods for the testing of three distinct groups of products were appended to the subcommittee's report. This report, after approval by the Councils of the two societies, was sent to the Minister of Agriculture who agreed, in January 1941, that it should be submitted to the manufacturers' organization, the Association of British Insecticide Manufacturers, as a basis of discussion.

The Association of British Insecticide Manufacturers gave rapid but full consideration to the report but, in late March 1941, reported that it was not satisfied that the necessity for such a scheme had been established, and that it could not accept the general principles of testing and registration. Further, the A.B.I.M. recorded that, in their view, any such scheme should be put forward by the Ministry, and that that submitted was too complex and probably too costly for consideration under war conditions. Subsequent negotiations were therefore handled by the Ministry and, in December 1941, a simplified scheme was sent to the A.B.I.M. Simplification was attained by the refusal to admit products of wholly secret composition and by the abandonment of a testing organization. After agreed modification to the scheme, the A.B.I.M. in May 1942 expressed their willingness to co-operate, and nominated their representatives on the Joint Panel appointed to give the requisite guidance to the Advisory Committee which is the official approving body nominated by and responsible to the Minister of Agriculture. The birth of the scheme was publicly announced in October 1942, thus ending a period of gestation inexcusably long unless the infant is well developed, vigorous and able quickly to bring official recognition of the better-known and, in war-time, indispensable proprietary product. But its further growth is clearly dependent on the goodwill of those concerned in its operation. It is to be commended, in particular, to those responsible for advising growers, for, in this scheme, is their first established, as distinct from *ad hoc*, collaboration with manufacturers of pest control products. For this reason I was disappointed to find that no provision had been made for the direct representation of our Association or of the Advisory Services on the organization. Such representation is not possible on the Advisory Committee, for its proceedings are private and its members responsible only to the Minister of Agriculture. Representation of all interests in the Joint Panel would raise the problem of numbers, and it was thought better to ask members of the Joint Panel to act in a representative capacity for those interests without direct representation. As our Association and the Advisory and Research Services have several members on the Joint Panel, the disadvantages of indirect representation should not hold.

Publicity problems

Though personal contact is, in my opinion, the royal road to the full application of biology to agriculture, the printed or written word is the best form of memorandum for the grower whether it be letter, leaflet, article in the local or technical press, or poster. The research worker is often required to present his results in a form comprehensible to the grower; the Advisory Officer contributes to his county's and agricultural press; the Ministry

arranges for the publication of bulletins and leaflets; the technical press seizes with promptness and foresight on all sources of reliable information. Yet there are many signs that the results of research do not get through, in proper perspective, to the grower. The leading article of the *Gardeners' Chronicle* for 12 Sept. 1942, for instance, anticipated a reduced yield of potatoes in many districts as a result of early blight attack and asked why, 'when the cause of the disease and a sound remedy in Bordeaux mixture are known', this knowledge is not applied by all potato growers? Further, the leader-writer insisted that 'here is an example of a serious insufficiency in our methods of research', and that what 'is needed in Agricultural and Horticultural research is less propaganda and more following up to discover what, if any, are the limits beyond which a scientific generalization ceases to be true or to be worth while putting into use'. The generalization that he has in mind is evidently that Bordeaux mixture is a remedy for potato blight, a belief which he shares with a firm of fungicide manufacturers who ill-advisedly permits its advertising department to proclaim that 'Perenox prevents potato blight'. I have been unable to trace the author of the alleged generalization that copper fungicides, applied correctly, will give a blight-free crop. The dangerous over-emphasis is apparent to all who know that the fungicide is for the control and not the prevention of blight. Its proper function is to reduce the spread of blight in the earthed-up and blight-liable crop until tuber formation is well advanced. Once tuber growth has reached the stage giving the best yield, the haulm should be destroyed by cutting, by blight or by acid. The use of the fungicide, at that stage, rather than prevent blight would favour blight infection of the tubers. Contrary to the leader-writer's contention then, the reasons for the non-universal use of copper fungicides against blight are known, but clearly the recommendations on blight control have become distorted in their passage from research to practice. Yet, so far as I am aware, attention has not before been drawn either to the errors of the leading article or to the dangers of the advertisement. Nor do I know from whom these corrections should have come; certainly not from myself except as evidence that, under existing arrangements, the findings of the applied biologist are not reaching the grower in a satisfactory manner. That this shortcoming is general is indicated by the formation of the Agricultural Improvement Council, to which body I would commend the appointment of a corps of intelligence or public relations officers. These officers should be journalists acquainted with both practical and scientific aspects, able to translate research into vivid workaday English, wise enough to avoid sensationalism and to seek expert advice on their scripts before publication. To foster mutual confidence, they should be permitted to move freely among growers, Advisory Officers and research workers. Their services would quickly prove indispensable in the preparation of popular articles, of leaflets, of posters and, when the time comes, of exhibits. But this liaison service is needed now when no preventable loss of crop can be permitted.

POST-WAR RESPONSIBILITIES

Attention must now be paid to the post-war problems which will confront the Association in the reconstruction inevitable when the war has been won. That a catastrophe of this order could have befallen civilization when it had seemingly reached a high stage of evolution is a sufficient condemnation of the pre-war system. That the aggressor nations thought victory possible by an exploitation of the resources of science is an impeachment of science itself. So many of us were immersed in our own absorbing subject and are still too dazed by the horrors of war to recognize what was lacking in pre-war science and what must be done to remedy past failings. Yet two thoughts stand clear: that science must protect itself from exploitation; that science must accept responsibility not only in safeguarding humanity against such misuse, but in ensuring that mankind shall profit to the full by the impact of science. Science must be welded to a common front to guide the application of science. No longer is it right that the scientist should be content with an abandonment of all interests save his subject. It is not enough that he should double the bounds of knowledge unless he shares in its application. If not able, by temperament or inclination, to help as an individual in the application of that knowledge to the service of humanity, he must share that responsibility as a useful member of an appropriate organization charged with the task of guiding public opinion in the proper use of that knowledge. Attempts to distinguish between pure and applied science cease then to be fatuous and become evil. A new meaning has been given to the second Law of our Association, that its object 'shall be to promote the study and advancement of all branches of Biology with a special reference to their applied aspects'.

Before science can shoulder its tremendous responsibility, methods must be found for the organization of individual effort in corporate manner. In that integration our Association will play its allotted part. If, therefore, in my subsequent remarks, I seem to depart from the title of my address, it is because our responsibilities are common to science as a whole and the particular development of the Association's activities must conform to the broad plan of advancement.

THE UNIFICATION OF SCIENCE

The formation of scientific societies has, in the past, been fortuitous and actuated by one or both of two motives: for the purpose either of aiding scientific discussion or of elevating the scientific profession. The first object is more easily attained when the

society is of small dimensions, hence, a tendency to limit membership to a narrow subject or to a small locality, a tendency counterbalanced by economic factors if the society aims at the dissemination of knowledge. Hence, in the publishing society, an amalgamation of interests is involved limiting the scope of the society for the advanced discussion of the particular subject and tending to segmentation because the individual member feels that his particular subject is swamped by the general. Your Council has tried to counter this tendency and to retain the active interest of all members by careful selection of the subjects of discussion at successive meetings of the Association, but it is a debatable point whether or not it is wise to continue this policy in view of the growth of the Association. Might it not be better to recognize that segmentation is, sooner or later, inevitable and therefore to be planned? This is no new problem and, in other circles, two solutions have been tried. The first, subdivision on a geographical basis, is feasible in the widely scattered society or where industry or subject is localized but hardly seems to meet our purpose so well as the second, namely, subdivision on a subject basis.

Each subject group should be given adequate executive powers to run with some degree of autonomy, but precise recommendations on its status and constitution would be out of place here. The range of any subject group, be it wood preservation, the viruses, plant propagation, apiculture or toxicology, would be determined by the number of members interested and by the energy of the promoters of the group rather than by prestated rule. But advantages follow if such a group covers the applied aspects of a particular branch of biology be it mycology, ecology, helminthology or entomology.

The immediate purpose of this suggestion is to provide for the growing preponderance of plant pathologists in the membership of the Association and to avoid the danger that those applied biologists whose interests lie in other directions may suffer neglect. The subject group is intended to provide a fulcrum on which the enthusiast in a border-line subject can exert pressure on his colleagues now reluctant to join our Association. These enthusiasts will share, with the Council, the responsibility that by their efforts subjects scantily represented in the Association shall receive due attention. By the extension of subject groups, our Association will more amply fulfil its object which, as stated above, concerns all branches of biology.

Beyond this immediate purpose there is a deeper motive which concerns science as a whole. I have suggested that each subject group should deal with the applied aspects of one particular branch of biology. Now the members of that group will also be members of the society concerned with the academic, as distinct from applied, aspects of that biological science. The subject groups will then serve to link together all biological societies into an organization capable of exerting a unified pressure, yet bound, not by rigid rule or order, but by common interests and responsibilities.

This topic has a special bearing on the suggestion made recently by W. C. Moore in his Presidential Address to the British Mycological Society. He considered that the future here of plant pathology and mycology would best be served by a fusion of the Plant Pathology Committee of the British Mycological Society and our Association into a Society or Association of Plant Pathologists. As this proposal comes within the terms of reference of a joint committee of the British Mycological Society and the Association, appointed to consider the means by which the future development of the study and practice of plant pathology in this country can best be furthered, it would be unwise for me to say more than that, in my opinion, the subject group could here be used to avoid the amputation of non-pathological members of our Association. To provide for collaboration with the academic societies, the field of plant pathology, in its widest sense, would need to be divided into a number of related subject groups, of which that concerned with mycology would form the link with the British Mycological Society. Similarly, the groups interested in applied entomology would ally the Association with the Royal Entomological Society; those concerned with nutritional disorders, with toxicology or with the physiology of disease, would by common membership bring the Association into closer touch with the several societies associated with botany and chemistry.

It is among the chemical societies that the widest experiment of co-operation has yet been made in the establishment, in 1935, of the Chemical Council. On this Council the three chartered societies, namely, The Institute of Chemistry, The Chemical Society and The Society of Chemical Industry, are represented. Despite war-time difficulties, the Council has gone far in ensuring the financial stability of the two publishing societies concerned and of the Chemical Library. Joint sectional meetings are now general and, through its subject groups, the Society of Chemical Industry maintains collaboration with societies of kindred interests. Many members of our Association would, I think, welcome an opportunity of sharing in the activities not only of the Food Group but of the newly formed Agriculture Group of the Society of Chemical Industry.

The subject group exists primarily because those of like interests wish to profit by a pooling of experience and ideas. My aim has been to show that it can be made to serve for the unification of science by counterbalancing the economic tendency of the larger publishing societies to lose contact. Can the second motive activating the formation of a society, that is, the elevation of the profession, likewise be used in the welding of a scientific front? The difficulties appear greater, for only scientists are con-

cerned who are professionally qualified. Among our own membership there are many who belong to no such professional organization, and for them an immediate problem is to decide whether or not those in existence, such as the Agricultural Education Association, the Association of Scientific Workers, or the Association of University Teachers, adequately meet their needs. A second difficulty is that those economic factors which tend to segregate the publishing societies operate with greater force between the professional societies which, for the protection of their members, have to build up financial reserves or which, in the apparent interests of their profession, have erected barriers which frustrate co-operation. Even within the chemical profession the difficulties have proved too great, and the proposal to form a National Federation of Men of Science, publicly discussed at a Conference organized by the Institute of Chemistry in 1923, came to nought, though it is perhaps remembered in the recently formed Joint Council of Professional Scientists. This Council has been established for the war and immediate post-war period, but it may well form the nucleus of a more permanent organization to provide for co-ordinated action in matters of common interest. But even if established on a permanent basis acceptable to professional biologists it is probable, from its present terms of reference, that the Council would be primarily concerned with professional matters, the furtherance of science itself being but incidental. Hence, though the professional societies would add weight and influence to the scientific front, the publishing societies, linked by the subject groups, would form the better basis for its organization.

The integration of the publishing societies to a body with executive powers is a problem for deeper discussion than the suggestion that two solutions are possible. The first imposes on the Royal Society the responsibility for first action on the score of its history and prestige. Moreover, the Royal Society has accepted not only the Government view that it is the figurehead of British science, but also the status of the National Academy of Science for Great Britain. A closer examination reveals, however, that the term 'Academy' has here been used in the scholastic sense. A census of its Fellows, excluding those of the medical profession, shows that seven of every ten elected since 1919 hold academic posts. With this membership and the limitation of the number of Fellows elected annually, its representation of science is biased and is likely to remain biased. Nor is confidence in the Royal Society as the executive of the science front increased by its history as the adhering organization to the International Council of Scientific Unions. This Council was formed, at the close of the last war, for the purpose of facilitating international co-operation in scientific work and functioned through International Unions in different branches of science. For each Union a committee for Great Britain, the National Committee, was formed for the promotion of that branch of science with which it is concerned. The National Committee for Biology, which includes two members nominated by each of the prominent publishing societies including our Association, has been inactive for at least the past twelve years.

The second suggestion, not exclusive of the first, is that the British Association for the Advancement of Science should take the lead, for it has given abundant evidence recently of its drive and its live interest in the social and humanitarian responsibilities of scientists and in the part science must play in post-war reconstruction.

To be more explicit at this stage would I feel run the risk of introducing controversy on minor matters which might mask the bold outlines of my suggestions on the post-war organization of science. As I have had thrice to divert from the main line of argument to discuss particular aspects of the problems confronting our Association, may I re-sketch those outlines: a basal unification by means of subject groups interlocking the publishing societies; an apical unification to a Science Council on which the sciences would be represented by election from the publishing societies. The extension of subject groups is the responsibility of the individual scientist fostered by societies of which he is a member; the initiation of the Science Council is a task for the Royal Society and the British Association. The extension from the national to the international scale would inevitably follow, bringing to life that Commonwealth of Science fashioned by the President of the British Association at the 1941 Conference on 'Science and World Order'.

THE LANGUAGE OF SCIENCE

The problems confronting organized science are many-sided, ranging from those requiring immediate post-war resolution to remoter projects of long-term research. The restoration of prosperity to war-stricken peoples; the fuller application of objectively determined knowledge on human needs, especially in relation to health, nutrition and housing; the mobilization and development of material and intellectual resources; these are among urgent problems which have already been discussed in conference and the press. I can now refer to two others only, the one internal, the other external.

First, that of language, the medium of thought exchange so familiar that little heed has been given to its importance as a scientific instrument. The need for precision has been recognized in the more specialized nomenclatures; national or international committees by periodic recommendation help to lessen the dangers of indefiniteness. But among the more general terms, more so those which become of everyday speech or popular use, new meanings or significances always arise to cloud, even to alter, the

original. Each of us has his own interpretation of the term 'Plant Pathology'; to me it covers the study of ill-health in plants, others would define it as the study of those diseases in plants due to parasites themselves plants. They would argue that the word 'plant' here governs the whole in the same way as, in the expression 'insect pests', the insects referred to are the pests and not the hosts. Such an argument is unacceptable except as evidence of the ambiguity which creeps inevitably into language and which, uncontrolled, is detrimental to the exchange of scientific ideas and incompatible with the discipline of science.

The remedy may involve the derivation of a new vocabulary, but, in my view, that matters not, for the need for new words arises daily in the evolution of science. Yet at present these words are introduced haphazardly, and according to the needs or whims of the individual scientist who may not have at hand the best advice on philological morphology. Some twelve years ago I was dissatisfied with the hybrid derivation of 'phytocidal', a word then becoming accepted to describe plant-injuring properties on the analogy of 'fungicidal'. My attempts to replace it in an article I then had on the stocks by the more specific 'folicidal' or the more general 'phytotoxic' were discarded as puristic. More recently the word 'synergism' has been applied to the case in which mixtures of poisons exhibit a toxicity greater than the sum total effect of the component poisons. But as synergism is an early Christian doctrine which cannot be held by the mixture, the contemporaneous word 'synergy' seems the better choice to describe this property. Again, the need for a new word may arise when usage of a long but cumbersome word association becomes frequent. How useful would be a word to cover 'methods for the prevention of disease and the control of organisms causing damage to plants'! I have myself borrowed the German 'Plant Protection' in spite of the vision it conjures up of rows of glass cloches.

For those reasons I would plead that the Science Council should set up a main committee with sub-committees in the different sciences, which, with philological representation, will extend the work on nomenclature to that of terminology in its broadest sense. Its task would be to guide the evolution of language and to add to its precision as an instrument of science. Its work should begin before an uncontrolled evolution has produced the chaos which, till now, has been the only prod to considerations of a standard nomenclature. Clearly also, it should have strong collaboration with authorities in other countries, for its recommendations should be heeded not nationally but internationally, for science knows no frontiers.

The widespread adoption of this suggestion would quickly reveal the advantages and difficulties of a common world language for science. Fortunately, scientific terminology is based on roots borrowed from Latin or Greek. Hence most technical terms are easily recognizable in any language employing the Roman alphabet. It is the everyday words which give trouble in translation and which would raise the greatest problems if science wished to speak a common tongue. It would, I think, be found that English is the most convenient language to adopt not only because of its preponderance in scientific literature but because it makes less use of grammatical inflexion and concord than other modern languages. The trend, in the history of languages, has been towards the abandonment of inflexion to express case, gender, number, person or tense, and, on this criterion, English is the most highly evolved of languages. This simplification is indeed still proceeding, the second person singular survives only in a few local English dialects, the subjunctive and many of the irregular verb forms can now be classed as archaic. Cannot the process be completed as far as it can be by a purposeful simplification of grammar? What would be lost by discarding the surviving concord of the added 's' of the third person singular of the present tense? Could not the vowel changes in the past tense and past participle of irregular verbs be abandoned in favour of the '-ed' of regular verbs? Of what use be the complications of the present indicative of that most-used verb 'to be'? These and other anomalies such as the irregular plurals and the inflected pronouns could vanish from scientific English, which to some would leave an initial aversion to 'bad grammar', but to others would have a refreshing bucolic flavour. But to those not of English speech, the change would be welcomed not only because of simplification but as evidence of the willingness of those whose good fortune it was to absorb the language unconsciously in childhood to contribute towards the derivation of a common tongue.

It is for this latter reason that I would plead also for a simplification of spelling, though this problem is so intertwined with that of pronunciation that it scares a novice like myself. It seems axiomatic that the oral and the written language should coincide at all possible points and, to my mind, the anomalies of spelling, though of etymological interest, have value only to the music-hall comedian. It is inconceivable that a better agreement than that holding at present is beyond attainment by the step-wise adoption of an agreed general plan which might, at first, affect only spelling. Agreement now among English-speaking scientists on the differences which have already arisen between the two sides of the Atlantic, such as 'sulphur' and 'sulfur', would deter future divergences more impeding to the growth of science.

A simplification of the scientific vocabulary would, on the other hand, seem a retrograde step, for language must be able to interpret the finest shades of meaning. Better would be a standard scientific dictionary extending defined nomenclatures to the limit, which is the suggestion of an earlier paragraph.

Further, the avoidance of idiom, other than scientific, in writing for scientific journals would go far to simplify translation and the realization of a common scientific English. To him who would argue that an English without idiom is lifeless, I would suggest that resort to idiom to freshen a sentence is a self-condemnation which has no place in scientific literature. From which it must be inferred that I distrust idiom, for it is vital to racy writing and natural speech and would doubtless survive the introduction of a universal language, a remoter prospect for which I hold no brief, yet which I mention to illustrate the profound influence on humanity of corporate action in science. If science is unable to achieve a common language, how distant is the prospect of a universal auxiliary language; if science can speak one tongue, that will surely grow to world-wide use.

THE ETHICS OF SCIENCE

A common language is but a thin thread of the ties which science can spin to reunite mankind. A stouter bond and a nobler task is the training of humanity in those principles to which science itself is dedicated and to which it owes all. The command over nature which science has unreservedly given to mankind and the present wealth of scientific knowledge are ascribable, first, to scientific method and secondly, to scientific ethics. The first category includes the assembling of all facts and all thoughts on the problem in hand, the formulation of possible relationships, the selection of the probable solution and its rigorous test by experiment. Surely this method of attack is applicable to many of mankind's problems beyond those of the recognized sciences. Mathematics and biology have already provided, in the newly developed methods of biometry, of physiology, of sociology and of psychology, powerful means for the destruction of political controversy, replacing shibboleth or prejudice by factual knowledge. In the reports of that untiring band of workers who have chosen the uninspired title of 'P. E. P.' are to be seen the beginnings of a socio-biology which merits careful nurture and vigorous support. But it is to scientific ethics that I must turn and, in this category, I would place the discipline of science, its insistence on an impartial exposition of the facts, on a complete disclosure of the evidence on which the proposed solutions are based, and on an unbiased discussion of discarded hypotheses. To the high standard of this discipline more than to the merits of method or technique is due the tremendous progress of science. Can there be any doubt that extended beyond science to human affairs it will raise civilization to divine heights?

Here then is the primary task of the Commonwealth of Science; to convert mankind to the methods and ethics of science, to train the young in the discipline of science, to instil into statesman and politician the spirit of science, to show commerce that there is more profit in the full application of science than in its exploitation. To this task there is the complement, the exposure and correction of decisions or actions contravening scientific ethics; to train mankind to shun those whose judgement is warped by ignorance or self-deceit, by wishful thinking or prospects of momentary personal gain. The scientist who knowingly suppresses evidence contrary to the hypothesis he is seeking to establish, commits intellectual suicide; yet the politician who intentionally exaggerates arguments in favour of his policy and decries those of the opposition is still allowed to sway public opinion. Deliberate misrepresentations are still commended as hard-headed business; tongue-in-the-cheek dishonesty is still praised as diplomacy or tact. The formidable list of everyday offences against scientific ethics reveals the magnitude of the task confronting science. The timid would be scared by it; the cynic would class it as idealistic or fantastic; but it would inspire those zealous in this fuller and revolutionary application of science for the benefit of humanity.

Clearly this revolution must be stepwise and the attack on the pre-war standards and prejudices must come from all angles. It will have repercussions on science itself of which at least two merit immédiate attention.

First, there must be a fundamental change in the status of the sciences in education. On this subject I cannot speak with authority, but it is apparent that the high value of the scientific training as a mental discipline is inadequately reflected in modern academic curricula. In part this failing is due to the survival of a mythical sphere of science qualified by some slogan such as 'science for its own sake'. The main function of this "pure' science is academic. Scientific research carried out for no purpose of immediate application has as its objects: the exercise of the ability to analyse and to correlate facts, to formulate and to test ideas; the acquirement of the habit of precise and correct reasoning; the appreciation of scientific ethics. Once this is realized, then will academic science accept its proper task in education. No longer will a scientific training be a mere preliminary to a life spent in the service of science but it will become indispensable as an intellectual discipline to all worthy of positions of responsibility and to all who ask for an education in the art and understanding of life.

Secondly, there must be a change in the attitude of the scientific hierarchy towards the political responsibilities of science. Sir Henry Dale, for instance, in his Presidential Address to the Royal Society in 1941, expressed fears for scientific freedom if science became entangled in politics. He foresaw these dangers: that if science were allowed to be used as a political weapon, it would become impossible to protect science itself from political pressure; that fundamental research having no immediate practical appeal would be allowed to fall into

arrears through relative neglect; that the rigid standards of true science would be relaxed by allowing the convenience of results for policy or for propaganda to enter into the assessment of their validity as evidence. No one will deny that such dangers exist; it is for this reason that I have borrowed from political terminology in applying the expression 'science front' to organized science. But to use these risks as reasons for inaction, as arguments for maintaining a complete aloofness from such mundane applications, is to share the cowardice but to lack the otherworldliness of the hermit. Could not these very arguments have been used against the application of science to industry? Look then to industrial science to get the dangers of contamination in proper perspective.

Has science suffered from industrial pressure? Yes, I can find one instance which seems unwittingly to have been accepted as a necessary consequence of the application of science to industry; it has at least been ignored by those who profess their guardianship of the liberty of science. A review of scientific communications reveals that it is apparently necessary for some authors to secure permission to publish from their employers. In many cases no doubt the acknowledgement denotes only the friendly co-operation of author and employer but, in other cases, it may imply that the employer claims a right of censorship. Any such claim is offensive to the spirit of science which demands full freedom of interchange of knowledge. The employer who enforces such a claim, and it would appear that our own Department of Scientific and Industrial Research is among those employers, must be made to realize that his own interests suffer by the suppression of information, the true worth of which can only be developed by publication. I can find but two sordid reasons for this restriction: one, that matter pertaining to an application for patent rights is legally required to be unpublished. If that is the law then repeal should be immediate. The second reason is that, by withholding information, the employer hopes to profit by his monopoly of that knowledge to a greater extent that he would in unrestricted trade. In other words, his intention is to deny his debt to the very science that made his trivial monopoly possible. His pocket-bound mind cannot perceive that his legitimate interests are served better by his meanest contribution to the broad flood of scientific progress than by his greatest effort to monopolize. Moreover, the employee blackmailed into the acceptance of this imposition, is forced to sully his trusteeship of his proud scientific inheritance. Yet beyond this gross exception I know of no loss of scientific freedom which has resulted from the entanglement of science in industry.

The second danger cited by the President of the Royal Society, that fundamental science would become neglected, is illusory, for who would maintain that fundamental research has suffered because it has no immediate commercial appeal?

The third danger, that of falling from scientific grace, is a temptation to all scientists whether academic or applied; to advance it as an excuse for monastic recluseness is a calumny against all scientists, unworthy of a leader of science whose abilities would be better employed in building a science front strong enough not only to withstand unwanted pressure, be it political or commercial, but to correct political and industrial offences against scientific interests and principles. Then will science intercede in human affairs with no fear of pollution but, by its contact, will elevate and purify.

This then is my message to you: Take up the task which this war has revealed; accept individually and corporately the responsibilities which science must shoulder in post-war reconstruction; join hands with your sister societies and, by your example, forge a Commonwealth of Science which by a fuller application of scientific principles and ethics will lift mankind beyond the reach of evil.

Nitrogen nutrition of the onion

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Culture experiments with onions in sand demonstrated that the best range of concentrations of nitrogen for production of bulbs is 16·48–32·96 p.p.m., concentrations above or below this range leading to reduced yields.

Introduction and experimental

The general methods employed in these culture experiments in sand have been discussed (Woodman, 1940). The variety of onion used was Unwin's Reliance, and experiments were conducted in waxed plant-pots (see Woodman, 1941). The concentrations of important and minor elements in the nutrient solutions have been given (Woodman, 1943, Table 1). The full figures are not repeated, but the nitrogen in p.p.m. varied as follows: A, 65·92; B, 32·96; C, 16·48; D, 8·24; E, 4·12; F, 0·41; and G, nil. The numerical data obtained, and their method of presentment and analysis, were described under the references quoted.

OBSERVATIONS AND DISCUSSION

The number of pots was 112, arranged in randomized blocks for the seven treatments (A–G) replicated sixteen times. The seed was sown 11 Jan. 1940, and germination had occurred by 3 Feb. The seedlings were singled to one per pot on 23 Feb., and the onions were harvested 8 Aug. 1940. Each culture received 24 l. of its appropriate solution in lots of 250 c.c. about three times a week.

Solution A (most nitrogen) gave the largest tops throughout; they were slightly chlorotic, possibly because of excess nitrogen. At harvest, the tops with A, B, and C were dying off, and the roots (taken, as in horticultural practice, to be the edible bulb plus associated fibre roots) were ripe. Plants with all other treatments were normal. Differences in yield, etc., are set out in Table 1.

Great deficiency or absence of nitrogen resulted in small tops. The fresh roots with B and C were statistically the largest. Those with A were definitely smaller, and, taken in conjunction with the slight chlorosis of the tops, it seemed that this concentration of nitrogen was prejudicial to both health and yield. The summaries of results for the fresh yields of the whole plants, and for the yields of dry matter, more or less bore out these conclusions. It may thus be asserted that from a commercial viewpoint, the yield of onion, the best range of concentrations of nitrogen for the onion under experiment was 16·48–32·96 p.p.m.

TABLE 1. *Summaries of results for nitrogen experiment with onions. Weight in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Tops, FW	SSS	20·17	13·98	11·97	11·88	5·26	1·77	0·33	9·34	1·840
		A>B=C=D>E=F=G								
Roots, FW	SSS	59·93	79·19	74·85	30·19	16·74	1·22	0·29	37·49	3·222
		B=C>A>D>E>F=G								
Whole plants, FW	SSS	80·10	93·17	86·82	42·07	22·00	2·99	0·63	46·83	3·758
		B>A>D>E>F=G; B=C; A=C>D								
Tops, DW	SSS	2·74	2·18	1·59	1·24	0·61	0·18	0·04	1·23	0·134
		A>B>C=D>E>F=G								
Roots, DW	SSS	7·14	10·25	9·46	3·91	2·02	0·15	0·04	4·71	0·400
		B=C>A>D>E>F=G								
Whole plants, DW	SSS	9·88	12·43	11·05	5·15	2·64	0·33	0·08	5·94	0·455
		B>A=C>D>E>F=G								
Top/root, FW	SSS	0·54	0·18	0·16	0·47	0·34	1·50	1·17	0·62	0·131
		F=G>A=B=C=D=E								
Top/root, DW	SSS	0·55	0·22	0·17	0·37	0·32	1·25	1·04	0·56	0·097
		F=G>A>B=C; F=G>B=C=D=E; A=D=E								
Tops, % moisture	SSS	80·25	80·98	80·23	88·84	87·68	87·59	88·36	84·85	0·249
		D=G>F>A=B=C; D>E=F>A=B=C; E=G								
Roots, % moisture	NS	88·24	87·08	87·24	87·01	87·84	87·72	87·48	87·52	0·305
Whole plants, % moisture	SSS	87·58	86·61	87·17	87·76	87·97	88·77	88·10	87·71	0·290
		F>A=D>B; F=G>B=C; E=F=G>B; A=D=E=G; A=C=D=E								

Table 1 shows that the data for ten of the summaries of results were very strongly significant (SSS) at the 0·1 % point, and that data for the moisture contents of the roots were not significant (NS). It also demonstrates that the fresh tops were heaviest with most nitrogen, those for the next three treatments being statistically equal (A>B=C=D).

The subtables for the two ratios demonstrated that deficiency of nitrogen resulted in a relatively increased growth of top, measured as fresh or dry matter. The moisture contents of the tops were also greater with deficiency of nitrogen, possibly because of the drier (riper) condition of the tops of the more mature plants with A, B, and C (D=G>A=B=C). The moisture content of the onions themselves was uninfluenced by differing concentrations of nitrogen (NS for this subtable).

I thank Mr J. F. N. Leonard for his help in this experiment.

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Behaviour of the fungus *Rhizoctonia solani* Kühn in the soil

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Rhizoctonia solani was found able to grow as a saprophyte through natural unsterilized soil. Its rate of growth under different soil conditions in glass tumblers was studied by the Rossi-Cholodny soil-plate method. Growth was most rapid at the lowest soil-moisture content tested, viz. 30% saturation, and was accelerated by forced aeration of the soil. The maximum distance to which mycelial growth could be supported on the food reserves of the agar inoculum alone was some 5 cm., as shown by extent of growth through tubes of moist sand, but in 23 days the fungus grew 21–24 cm. through tubes of soil. Removal of the agar disk 2 days after inoculation of the tubes reduced growth through sand by more than half, but through soil by only a small proportion. In soil, *Rhizoctonia* was able to cause 100% damping-off of radish seedlings planted at a radial distance of 4 cm. from the agar inoculum, and some 40% damping-off at a distance of 9 cm. The depressing effect of additions of 1% ground-wheat straw or dried grass to the soil upon growth of the fungus was attributed to (1) the negligible cellulose-decomposing ability of *Rhizoctonia*, (2) nitrogen starvation of the mycelium, through rapid utilization of the available soil nitrogen by the cellulose-decomposing micro-organisms multiplying upon the fresh organic material, (3) fungistatic action on *Rhizoctonia* of the respiratory carbon dioxide produced by the cellulose-decomposers.

Introduction

In an ecological survey of the root-infecting fungi, Garrett (1938*a*) distinguished the less specialized soil fungi which still retain at least a limited capacity for free and independent growth through the soil, from the more specialized root parasites which can only spread through the soil along the underground parts of their host plants. As a typical representative of the former group, Garrett cited *Rhizoctonia solani*, which 'may be microscopically observed to make at least a limited hyphal growth through the soil'. Owing to the economic importance of this fungus, much is known of its physiology in pure culture and its behaviour as a parasite under a wide range of soil conditions, but whilst a few investigators (Sanford, 1938) have studied the saprophytic growth of the organism in sterile soil, none has so far reported any experiments on the saprophytic growth of the fungus in *natural unsterilized soil*. Although sterilized soil is a medium less far removed from natural soil than the agar plate, it is yet an unsatisfactory substitute for the natural environment. The object of the present investigation was to study the behaviour of *R. solani* in natural unsterilized soil using the Rossi-Cholodny 'soil-plate method'.

A fresh impetus was given to the science of soil microbiology by the formulation of methods for direct microscopical study of the soil micro-organisms (see Conn, 1932). Conn (1917) first reported systematic observations on soil infusions, made in dilute gelatin, dried on a slide, and stained with phenolic rose bengal or erythrosin, and his study was followed by those of Winogradsky (1924 *a*, *b*, 1925). Conn's method was improved by Rossi (1928) and Rossi & Gesuè (1930), who exposed a vertical face of soil, against which was pressed a clean slide. By fixing and staining, a picture of the micro-organisms was obtained with a minimum of disturbance. This technique was improved by Cholodny (1930), who left the slides in position for a period of days or weeks before removing, fixing and staining for examination. The potentialities of the Rossi-Cholodny technique were demonstrated by Conn (1932), in a study of micro-biological changes occurring in differently treated soils in glass tumblers. Whilst soil microbiologists have put the Rossi-Cholodny technique to various uses, King *et al.* (1934) are the only plant pathologists to report use of the method, in a study of microbiological changes following heavy applications of organic manures to a soil infected with *Phymatotrichum omnivorum*, the agent of root rot of cotton in the U.S.A. Both *P. omnivorum* and *R. solani* are characterized by mycelium with easily recognizable morphological peculiarities, which is almost an essential for this particular use of the Rossi-Cholodny technique.

Sanford (1941) showed that the conditions favouring maximum saprophytic activity of *R. solani* in the soil might not be optimum for its parasitic activity. He found that whilst the growth of mycelium over the surface of potato sprouts was more copious in steam-sterilized soil than in unsterilized soil, the extent and intensity of infection was much less, thus confirming an earlier observation by Richards (1921). If pure culture inoculum of *Rhizoctonia* grown on steam-sterilized soil was mixed with unsterilized soil in proportions ranging from $\frac{1}{16}$ to $\frac{3}{4}$ of the mixture, the virulence of the fungus was greatest where the amount of inoculum was least. Sanford tentatively concluded 'the pathogen is most virulent when its

* This investigation, which was carried out during the writer's tenure of the Macmillan Brown Agricultural Research Scholarship of the University of New Zealand, 1939–41, was completed at the Dominion Laboratory of Plant Pathology, Winnipeg, Canada.

hyphae are very young, very thin and still hyaline, and difficult to find on the susceptible host tissue. Indeed, when the mycelium can be easily seen and especially when it masses more or less heavily, susceptible host tissue is usually afforded complete protection from infection (Fig. 2 B) despite the fact that the isolate is potentially very virulent. This observation has been so consistent in these experiments during seven years that one must conclude that factors which cause massing of the mycelium also depress its virulence.'

METHODS

The mode of using the Rossi-Cholodny technique was in essentials that of Conn (1932); in the preparation and handling of soils Garrett's methods (1938*b*) were followed. In the majority of the experiments, glass tumblers were used as soil containers. Soil was air-dried and passed through a 2 mm. mesh sieve before use; saturation capacities for moisture were determined by the perforated box method of Keen & Raczkowski (1921). For soil-moisture contents of less than 50% saturation, soil and water were mixed by hand before the tumblers were filled; for moisture contents of 50% saturation or above, the air-dry soil was weighed into the tumblers and water added later, after the four microscope slides had been placed in position. Inoculation of the soil with *R. solani* was always left at least until the day following addition of water to the soil. For inoculum, 6 mm. disks were cut with a cork-borer from the growing margin of a colony on potato-dextrose agar; a single disk was placed in contact with the inner face of each glass slide, 1 cm. below the soil surface. After inoculation, tumblers were covered with a mulch of moist sand, at the same degree of saturation as the soil. Losses in weight through evaporation of water were made good by periodical weighing and addition of distilled water to the tumblers. A minimum of three tumblers, giving not less than twelve slides, was provided for each soil type or treatment in any experiment.

The slides were examined according to the directions of Conn (1932), i.e. the core of soil containing the slides was first removed from the tumbler, and then each slide was pulled carefully away from the inner face of soil; in this way, the 'picture' was not damaged by any shearing of the slide with the soil. After air-drying of the slide, the micro-organisms were fixed and stained by a 1 min. immersion in 5% erythrosin in 5% aqueous phenol maintained at 100° C. in a boiling water-bath. Excess stain was removed by washing in tap water and then in distilled water, and the slide was then air-dried. Subsequent microscopical examination was made under a coverglass and water mount.

The growth of *Rhizoctonia* through the soil along the glass surface of the slide was followed under the microscope from the inoculum to the tips of the most advanced hyphae. This latter point was marked on the slide, and the distance between the two marks measured. Other micro-organisms, including soil fungi, also developed on these slides, but it was not difficult to distinguish between hyphae of the inoculant and those of adventitious fungi; the hyphae of the inoculant could be traced in almost unbroken connexion from the inoculum to the advancing margin of the colony. Moreover, the hyphae of *R. solani* possess morphological peculiarities which aid recognition. It may be objected that the rate of growth of hyphae along a glass slide might not always be the same as that through the network of soil particles. This cannot be denied, yet nevertheless it is contended that the Rossi-Cholodny method affords at least a *comparative* index of the growth rate of *Rhizoctonia* under different soil conditions.

EXPERIMENTAL

The following isolates of *R. solani* were employed: R_1, R_2, R_3 and R_4 obtained from sclerotia on potato tubers, and R_5 and R_6 isolated from the roots of wheat plants from Norfolk, where *R. solani* causes a disease of cereals considered by Dillon Weston & Garrett (1943) to be identical with that reported by Samuel & Garrett (1932) from South Australia and by Hynes (1937) from New South Wales. Isolates R_9 and R_{11}, from roots of lettuce and radish, respectively, were supplied by Dr I. F. Storey, and isolates S_{21}, S_{22}, B_{140}, and M_2 were isolated in Canada from stem lesions on wheat plants (a condition not to be confused with the Australian and Norfolk disease, which affects the roots but never the stem).

(*a*) *Influence of soil-moisture content upon the growth of* Rhizoctonia

Five isolates of *Rhizoctonia*, R_1, R_2, R_5, R_9 and R_{11}, were employed in turn in this series of experiments, in which were studied the effect of soil-moisture content upon hyphal growth in four types of soil, viz:

Harwood: a clay loam, *p*H 6·8, water-holding capacity 43%.

Allotment: an organic loam, *p*H 7·0, water-holding capacity 47%.

Woburn: a sandy loam, *p*H 6·4, water-holding capacity 28%.

Sand: a quartz sand, *p*H 6·8, water-holding capacity 22%.

Three moisture contents, viz. 33, 50 and 80% saturation, were compared in this series of experiments; three tumblers were held at each moisture content, so that mean growth estimates are derived from measurements of twelve slides. Slides were examined 5 days from the start of the experiment (Table 1).

Most growth was always made at the lowest soil-moisture content, and least growth at the highest. This reduction in growth can be attributed to a

TABLE 1. *Growth of* Rhizoctonia (*in mm.*) *in various soils*

Soil type ...	Harwood			Allotment			Woburn			Sand		
Moisture content*	33	50	80	33	50	80	33	50	80	33	50	80
R_1	43	13	0	52	44	11	50	45	7	33	20	23
S.E.	±1·4	±0·7	—	±0·5	±0·4	±4·0	±0·6	±0·9	±0·8	±2·3	±0·7	±2·1
R_2	16	12	0	49	15	6	48	45	2	35	27	23
S.E.	±2·3	±2·0	—	±0·3	±0·3	±1·0	±0·7	±0·9	±0·1	±0·3	±0·7	±2·0
R_5†	46	29	4	42	19	2	41	19	5	23	17	13
S.E.	±1·9	±1·9	±1·9	±1·9	±1·9	±1·9	±1·9	±1·9	±1·9	±1·9	±1·9	±1·9
R_9	51	36	0	50	15	7	41	38	3	27	20	17
S.E.	±5·1	±3·5	—	±0·1	±2·8	±0·1	±3·0	±4·0	±2·0	±2·6	±3·8	±4·9
R_{11}	53	35	2	38	12	0	18	13	2	25	22	16
S.E.	±2·1	±4·9	±0·9	±5·4	±2·2	—	±3·4	±2·3	±0·1	±1·6	±1·5	±1·7

* As percentage saturation.

† For results of isolate R_5, standard errors calculated by analysis of variance method, but for the remainder by the root mean square method.

decline in soil aeration with rise in moisture content; in sand, where aeration is better than that in any soil, growth of the fungus is least affected by rise in soil-moisture content.

(*b*) *The effect of soil aeration and texture upon growth of* Rhizoctonia

In order to test the hypothesis that variation in soil-moisture content over the range 33–80 % saturation influenced the growth of *Rhizoctonia* chiefly through its effect on soil aeration, the effect of forced aeration of the soil was examined. Garrett's (1937) method of aerating the soil was employed; the soil containers were wide-necked glass bottles connected in series with an air pump, which forced some 350 c.c. of air/min. through the soil. Slides were also set up in similar containers not connected to the pump, to serve as non-aerated controls. The allotment soil chosen for these experiments was brought to a moisture content of 50 % saturation; isolates R_1 and R_5 were employed as inoculants in consecutive experiments, the results obtained after 6 days' growth being given in Table 2.

TABLE 2. *Growth of* Rhizoctonia (*in mm.*) *in aerated and non-aerated soils*

	Aerated	Non-aerated
R_1	31	20
S.E.	±2·8	±3·2
R_5	28	19
S.E.	±1·2	±1·9

In each experiment, forced aeration of the soil significantly increased growth of *Rhizoctonia*.

The converse experiment was next done, viz. an attempt to retard the natural aeration of sand, by incorporation of calcium bentonite in proportions ranging from 2 to 10 %. The moisture contents of these sand-bentonite mixtures were adjusted to 40 % saturation. The results after 5 days' growth, in experiments with isolates R_1 and R_5 in turn, are given in Table 3.

The admixture of bentonite with sand had the expected effect of significantly reducing growth of the fungus; there were no significant growth differences between the different sand-bentonite mixtures. The *p*H value of all sand-bentonite mixtures was 7·2, whereas that of the untreated sand was 6·8;

TABLE 3. *Growth of* Rhizoctonia (*in mm.*) *in sand ± bentonite*

% bentonite with sand ...	0	2	5	8	10	S.E.
R_1	25	16	17	17	15	±1·2
R_5	30	14	15	14	12	±3·1

this slight alkalinity is unlikely to have reduced growth of *Rhizoctonia* by any appreciable amount (see under).

(*c*) *The effect of soil reaction upon growth of* Rhizoctonia

A series of soils of different reaction was prepared by treatment of allotment soil with suitable amounts of calcium hydroxide and sulphuric acid, respectively, and subsequent incubation of the moist treated soils for a period of 3 weeks. The soils were then air-dried, brought to a moisture content of 40 % saturation, and weighed into the tumblers. Growth made by the isolates R_1 and R_5, in consecutive experiments, is recorded in Table 4, together with *p*H values of the soils at the beginning and end of the 5-day fungal-growth period.

Good growth was made by both isolates over the range *p*H 5·8–8·1 in these experiments; a neutral reaction was optimum for growth of the organism.

(*d*) *Linear extent of mycelial growth from the inoculum*

The experiments so far described demonstrate the influence of soil conditions upon the rate of growth of *Rhizoctonia* through the soil but do not indicate whether the food supply is drawn entirely from the agar inoculum, or whether this is supplemented, or

the fungus even wholly supported, by nourishment obtained from the soil. Since a sufficient range of growth was not obtainable on the glass slides in the tumblers, a similar experiment was carried out in glass tubes 30 cm. long and 1·9 cm. internal diameter. They were filled with dry allotment, Harwood, or Woburn soil or quartz sand, as employed in the experiments on soil-moisture content; twelve tubes were allotted to each soil type. Water was then added in sufficient quantity to bring the moisture contents to 50% saturation and sufficient time allowed for the water to distribute itself through the soil before inoculation of one end of the soil column with a disk of agar inoculum of *Rhizoctonia*, 1·9 cm. diam. A further plug of the same moist soil as that in the rest of the tube was placed between the inoculum disk and the extremity of the tube to check drying the agar. Daily measurements of mycelial growth along the walls of the tubes were made under the dissecting microscope by reflected light (Table 5).

TABLE 4. *Growth of* Rhizoctonia (*in mm.*) *in soil adjusted to different reactions*

*p*H of soil	3·0	4·5	5·8	6·3	7·1	7·4	8·1	8·7
R_1	0	1·4	34	35	45	32	29	5
S.E.	—	±0·8	±3·4	±1·8	±0·6	±0·8	±0·9	±0·6
*p*H of soil	3·0	4·7	5·9	6·3	7·0	7·4	8·1	8·9
R_5	0	9	24	25	32	28	24	4
S.E.	—	±2·0	±2·1	±2·1	±2·0	±2·0	±2·1	±2·0

TABLE 5. *Growth of* Rhizoctonia (*in mm.*) *through soil columns*

Days after inoculation	2	6	8	13	15	18	21
Harwood soil	16	46	67	109	122	167	196
Allotment soil	17	59	89	129	143	155	172
Woburn soil	10	54	74	125	152	195	228
Sand	12	33	37	51	52	52	52
	S.E. ±4·6						

The differences in extent of growth of the fungus through allotment, Harwood and Woburn soils during the experimental period of 21 days were not great, but in the quartz sand growth ceased after 13 days, when the hyphae had travelled some 5 cm. from the inoculum. It is concluded, therefore, that the additional 12–17 cm. growth made in the three soils was supported by nourishment derived from the soil itself. This conclusion was tested in another way. An experiment was set up in glass tubes, employing the same four soils and similar to the first one in every respect save type and treatment of the inoculum. Twelve tubes were filled with each type of soil; in four tubes the disk of agar inoculum was left undisturbed throughout the experiment, in four tubes the agar inoculum disk was removed after 2 days, and in four tubes the inoculum was provided not by an agar disk but by mycelial strands picked off with a needle from the vertical sides of a Petri dish. Frequent measurements of growth were made as before (Table 6).

Removal of the agar inoculum disk after 2 days, or the substitution of mycelial strands as inoculum, diminished growth of *Rhizoctonia* greatly in the sand tubes. In the three soils, removal of the agar inoculum disk reduced growth by only a slight amount. In Harwood soil, growth from the mycelial strands almost caught up with that from the agar disks after 23 days, but in allotment and Woburn soils the extent of growth from the strands was only about half that from the agar disks at 23 days. A certain 'food potential' of the inoculum may thus be necessary to initiate, though not to maintain, saprophytic growth of *Rhizoctonia* through the soil. Garrett (1938*a*) drew attention to the need of a 'food base' by certain fungi before they can establish a root in-

TABLE 6. *Influence of the inoculum upon growth of* Rhizoctonia (*in mm.*) *through soil columns*

Days after inoculation ...	3	6	8	11	14	16	18	20	23
Harwood soil:									
Agar inoculum left	20	61	77	93	127	173	180	199	237
Agar inoculum removed	19	51	60	69	120	146	151	183	224
Mycelial strand inoculum	8	27	41	55	71	118	133	146	191
Allotment soil:									
Agar inoculum left	27	60	89	118	134	169	180	203	214
Agar inoculum removed	26	62	88	114	126	139	152	170	179
Mycelial strand inoculum	8	33	55	77	78	87	96	100	105
Woburn soil:									
Agar inoculum left	20	58	79	100	129	160	172	194	220
Agar inoculum removed	19	55	76	97	128	149	151	173	192
Mycelial strand inoculum	10	27	41	55	67	85	90	100	123
Sand:									
Agar inoculum left	15	30	32	39	41	41	41	41	41
Agar inoculum removed	14	16	18	19	19	19	19	19	19
Mycelial strand inoculum	5	6	6	6	6	6	6	6	6
			S.E. ±16·0.						

fection; once such an infection has been started, the fungus becomes independent of the food base provided by the original inoculum.

During the course of these two experiments with soil tubes, the development of sclerotia against the glass walls was observed. Sclerotia were counted during the first experiment, numbers reaching a maximum within 6 days of inoculation in all tubes: no sclerotia formed farther than 6 cm. from the inoculum (see Table 7).

TABLE 7. *Numerical distribution of* Rhizoctonia *sclerotia in soil tubes*

Distance from agar inoculum disk (cm.)	1–2	3–4	5–6	7–8
Harwood soil	30	5	4	0
Allotment soil	40	16	3	0
Woburn soil	50	12	2	0
Sand	3	4	0	0

Formation of sclerotia evidently depends upon a copious supply of nutrients from the inoculum, since no sclerotia formed farther than 6 cm. away from the agar disk; no sclerotia were formed when the mycelium of the fungus reached the other end of the tube, where aeration conditions would have been comparable to those in the vicinity of the inoculum. The sparse production of sclerotia in sand, as compared with that in the soils, may perhaps be ascribed to a scarcity of inorganic nutrients; in their experiments on the production of sclerotia by *R. solani* on a synthetic nutrient medium, Tyner & Sanford (1935) found minimal and optimal requirements for phosphorus were 7·5 and 31 p.p.m., and for nitrogen 50 and 560 p.p.m.

Finally, the ability of *Rhizoctonia* to make a free saprophytic growth through the soil was tested by determining its ability to cause damping-off of radish seedlings sown at a range of distances from the agar inoculum disk. The soils employed were allotment and Harwood, passed whilst moist at the time of collection through a ¼ in. sieve, and then brought to a moisture content of 40% saturation before weighing into circular cake-tins of 19 cm. diam. and 8 cm. deep. An agar inoculum disk, 1·5 cm. diam. of isolate R_{11} from radishes, was buried a few mm. below the soil surface in the centre of each tin. A ring of radish seed was then sown at the required radius from the inoculum disk, the radii of the rings being 1–9 cm. As the experiment was carried out in unsterilized soils, control tins were planted without inoculum disks to serve as a check on the possible occurrence of damping-off fungi already in the soil, but no case of damping-off occurred in any of the control tins. Moreover, every seedling recorded as damped-off in the inoculated tins was examined microscopically, to confirm the presence of *Rhizoctonia*. After planting, the tins were kept in diffuse light in the laboratory. Percentage pre-emergence killing was calculated from the difference between emergence in control and in inoculated tins; records of damping-off were made at short intervals (see Table 8, where each figure represents the mean for four individual tins).

As was expected, the percentage of pre-emergence killing tended to decrease as the distance of seeds from the inoculum disk increased. The nearer the ring of seedlings to the inoculum, the earlier and more extensively did damping-off occur; in both soil types, 100% of seedlings eventually damped-off at the 4 cm. distance from the inoculum. At distances greater than this, the percentage of damping-off did not reach 100, though even at the 9 cm. distance some 40% of seedlings eventually damped-off in each soil.

TABLE 8. *Percentage damping-off of radish seedlings by* Rhizoctonia *at a distance from the inoculum*

Soil type	Distance of seedlings from inoculum (cm.)	% pre-emergence killing presumed due to *Rhizoctonia*	% damped-off seedlings after (days)					
			7	8	9	11	13	16
Harwood	1	4	31	76	100	100	100	100
	2	4	11	19	45	91	100	100
	3	2	2	6	8	55	77	100
	4	5	10	24	31	45	72	100
	5	0	0	0	7	24	63	83
	6	0	0	0	0	5	42	70
	7	0	0	4	16	28	36	72
	8	1	0	0	5	12	29	52
	9	0	0	0	0	13	20	41
Allotment	1	25	91	100	100	100	100	100
	2	15	34	63	75	94	100	100
	3	13	0	6	14	35	60	100
	4	8	20	43	70	85	93	100
	5	3	6	37	60	72	86	95
	6	3	8	39	54	63	79	90
	7	0	0	10	31	35	39	58
	8	0	0	22	32	33	36	56
	9	0	0	8	10	15	26	42

Failure of *Rhizoctonia* to cause 100% damping-off at distances exceeding 4 cm. from the inoculum need not necessarily be interpreted as due to lack of parasitic vigour in the fungus; it is equally explicable by the assumption that the seedlings had passed through the phase of greatest susceptibility to damping-off before the fungus reached them.

Essentially similar results were obtained in a second experiment of a similar type, the same two soils being employed, but at each of two moisture levels, 33 and 50% saturation. In each soil, the progress of damping-off was more rapid at the lower soil-moisture content, in agreement with behaviour of the fungus on the Rossi-Cholodny slides.

partially decomposed materials, farmyard manure and Adco compost, produced a smaller depression, whilst the city refuse compost depressed growth least.

In a second experiment, the addition of 1% of wheat straw meal to Red River soil, a Chernozem black clay loam (*p*H 7·0) from Manitoba, was tested for its effect on the growth of five isolates of *Rhizoctonia* from wheat. The soil was maintained at a moisture content of 40% saturation and mycelial growth along the slides was measured after 12 days (Table 10).

The addition of the wheat-straw meal to the Canadian soil depressed the growth of *Rhizoctonia*

TABLE 9. *Growth of* Rhizoctonia (*in mm.*) *in soil with organic supplements*

	In Harwood soil. Growth after (days)			In allotment soil. Growth after (days)			In Woburn soil. Growth after (days)		
	6	12	18	6	12	18	6	12	18
No soil supplement	35	47	65	37	40	51	34	37	65
+1% grass meal	12	14	16	15	15	16	13	10	10
+1% farmyard manure meal	19	28	38	23	24	28	14	20	19
+1% Adco compost meal	22	24	31	24	36	39	14	16	29
+1% city refuse meal	31	35	44	24	34	40	21	23	42
S.E.	±2·2	±2·0	±3·0	±2·2	±2·0	±3·0	±2·2	±2·0	±3·0

(*e*) *Influence of organic supplements upon the growth of* Rhizoctonia

The foregoing experiments seem to have established the ability of *Rhizoctonia* to grow through the soil as a saprophyte, but this does not necessarily imply that its growth will be encouraged by the addition of fresh organic matter to the soil, or that such additions must lead to a subsequent extension of its parasitic activity. The addition of any type of organic material to soil encourages the development of a microflora best fitted to decompose that particular material; the activity of the other soil micro-organisms is apt to be depressed, at least for a time, below normal level.

(i) *The depressing effect of certain organic supplements.* In a first exploratory experiment, four organic materials in the form of dried ground meal, viz. grass, farmyard manure, Adco compost, and city refuse compost, were compared in allotment, Harwood and Woburn soils. The meals were added to the soil in the proportion of 1% by weight in this and subsequent experiments; the moisture-holding capacity of each mixture was determined separately, since this capacity was slightly increased by addition of the organic supplements. None of the organic materials produced more than a trivial change in the *p*H value of the soil. Results, based on measurements of twelve slides from each series are given in Table 9.

In each of the soils, all four organic materials depressed growth of *Rhizoctonia*. The greatest depression was produced by grass meal, which has the highest content of readily decomposable matter; the by an amount comparable to that due to addition of grass meal to the three English soils. A similar effect both with wheat-straw meal and with grass meal was obtained in a second experiment with Red River soil.

TABLE 10. *Growth of* Rhizoctonia (*in mm.*) *in soil + 1% straw meal*

Rhizoctonia isolate ...	S_{21}	S_{22}	B_{140}	M_2	R_5
No soil supplement	32	32	30	26	24
+1% straw meal	15	12	12	12	13
S.E. ±1·1					

(ii) *Removal of depressing effect of organic supplements by soil sterilization.* The most obvious reason for the depressing effect of grass and wheat-straw meal upon the growth of *Rhizoctonia* was apparent on a cursory examination of the slides—the organic supplements had fostered a profuse development of other soil micro-organisms. The effect of these additions of organic matter was, therefore, compared in sterilized and in unsterilized Red River soil. In addition to wheat-straw and grass meals, lucerne meal was employed, each being added in the proportion of 1% by weight to the soil. The experiment was carried out in 4 in. unglazed clay pots at a soil-moisture content of 40% saturation. Sterilization of the soil and organic matter was carried out (*a*) together, by autoclaving for 3 hr. at a pressure of 1 atm., (*b*) separately, the soil being autoclaved as in (*a*), but the organic meals being autoclaved at the same pressure for 20 min. to avoid unnecessary heat change in the organic meals during sterilization. The subsequent mixing of the sterile soil and organic meals, addition of sterile

water, and any further handling, were done in a pure culture chamber: the inoculant was R_1 (Table 11).

The depressing effect of the organic supplements upon growth of *Rhizoctonia*, which was again manifested in the unsterilized soil, failed to appear in the sterilized soil. Sterilization of the soil without organic supplements, however, increased growth of the fungus only slightly.

TABLE 11. *Effect of soil sterilization upon growth of* Rhizoctonia *(in mm.) in soil with organic supplements*

	Treatment of soil + organic materials					
	Not sterilized		Mixed and then sterilized		Sterilized and then mixed	
Growth period of *Rhizoctonia* (days) ...	6	12	6	12	6	12
No soil supplement	32	40	35	53	30	46
+ 1 % grass meal	11	12	37	54	32	43
+ 1 % lucerne meal	13	14	38	57	31	43
+ 1 % straw meal	12	13	39	57	30	42
S.E.	± 2·0	± 1·9	± 2·0	± 1·9	± 2·0	± 1·9

In a second experiment, an endeavour was made to induce this depressant action of the organic supplements upon growth of *Rhizoctonia* in steam-sterilized Red River soil, by inoculation of the sterilized soil with a mixed fungus inoculum. For the preparation of suitable inocula, batches of sterile soil at a moisture content of 40 % saturation were inoculated with two mixtures of fungi, viz.: A: *Penicillium terrestre*, *P. intricatum*, *Aspergillus flavipes*. B: *Gliocladium roseum*, *Rhizopus elegans*, *Trichoderma viride*.

The inoculated soils were incubated at 20° C. for 10 days before use. In this experiment, soil and organic meals were sterilized separately; since lucerne meal had produced a very similar growth depression to that of the other two organic meals in the previous experiment it was omitted from this. The fungus inocula were mixed with the different soils in the proportion of 1 part of inoculum to 6 parts of soil. For the sake of completeness of experimental design, all treatments of sterilized soil were matched by similar treatments of unsterilized soil. Isolate R_1 was the inoculant and growth was measured after 12 days, on twelve slides from each series (Table 12).

Both inocula reduced growth of *Rhizoctonia* in the sterilized soil and organic meal mixtures to the level of that in the unsterilized mixtures, but in the soil without organic supplements, sterilization failed to increase growth. Addition of the fungus inocula, on the other hand, significantly depressed growth both in the sterilized and in the unsterilized soil without organic supplements, the effect being greater in the former.

These two experiments demonstrated that the depressing action of the organic supplements upon growth through unsterilized soil is a microbiological effect. In order to analyse this 'antibiotic' effect of other micro-organisms upon the growth of *Rhizoctonia* somewhat further, three lines of investigation have been pursued:

(1) An examination of the cellulose-decomposing ability of *Rhizoctonia*, in order to estimate the degree of its disadvantage, when thrown into competition with active cellulose-decomposers, encouraged by addition of the cellulose-rich organic supplements to the soil.

(2) An inquiry into competition between the cellulose-decomposing micro-organisms, multiplying on the organic supplements, and *Rhizoctonia* for supplies of essential nutrients, such as nitrogen, from the soil.

(3) A study of the influence of carbon dioxide upon the growth of *Rhizoctonia*, in order to evaluate the effect of intensified soil respiration, following upon addition of organic supplements to the soil.

TABLE 12. *Effect of soil sterilization and reinoculation upon growth of* Rhizoctonia *(in mm.) in soil with organic supplements*

	Soil not sterilized		
	No inoc.	Inoc. A	Inoc. B
No soil supplement	41	26	28
+ 1 % grass meal	15	14	12
+ 1 % straw meal	15	13	13
		S.E. ± 1·8.	

	Soil sterilized		
	No inoc.	Inoc. A	Inoc. B
No soil supplement	42	21	22
+ 1 % grass meal	39	14	16
+ 1 % straw meal	40	15	15
		S.E. ± 1·8.	

(iii) *The cellulose-decomposing ability of* Rhizoctonia. No experiments upon the cellulose-decomposing ability of *R. solani* seem to have been reported; such experiments were therefore carried out by two different methods. In the first experiment, the cellulose-agar plate method of McBeth (1916), as modified by Sanborn (1927), was employed. By this method, precipitated cellulose and agar are added to a mineral nutrient solution together with an indicator, china-blue aurin; the growth of an active cellulose-decomposer on the plate changes the colour of

the medium from grey to deep blue. Thirteen isolates of *Rhizoctonia* were grown on these plates in comparison with eight other fungi, selected as typical cellulose-decomposers; three plates were inoculated with each isolate, and results were recorded 7, 10 and 15 days after inoculation, according to the following scale:

− no reaction.
+ a slight positive reaction on 1 plate.
++ a slight positive reaction on 2 plates.
+++ a distinct positive reaction on 3 plates.

The results are given in Table 13.

(iv) *Mitigation of the depressing effect of organic matter by supplementary nitrogen.* The foregoing experiments suggested that the poor growth of *Rhizoctonia* in soil to which fresh grass or straw meal has just been added may be due to the activity of the cellulose-decomposing microflora. One consequence of such activity would be an absorption of the readily available nitrogen of the soil by the cellulose-decomposers; the possible effect of the nitrogen poverty thus induced in the soil upon the growth of *Rhizoctonia* was therefore tested in the following experiments. The soil employed was of the Red River stock, with total nitrogen 0·35 %; the nitrogen contents of the grass and wheat-straw meals were 4·12 and 1·62 % respectively.† Of the dried organic meal 1 % was mixed with the soil; supplementary nitrogen was added at the rate of 12·5 mg. N/100 g. air-dry soil, i.e. 1·25 g. N/100 g. grass or straw meal. This amount would have been amply sufficient for complete decomposition of the straw meal (the poorer in nitrogen of the two supplements) even in the complete absence of soluble nitrogen from the soil. The soil was brought to a moisture content of 40 % saturation. Replication was at the rate of three pots, or twelve slides, for each treatment at each of the two samplings (Table 14).

TABLE 13. *Cellulose-decomposing ability of different* Rhizoctonia *isolates and of some other fungi by the Sanborn-McBeth method*

Isolates ...	M_2, R_6	R_1, R_2, R_{10}	R_8	S_{31}	R_{11}, S_{21}, S_{23}	S_{30}	S_{22}, S_{25} *Penicillium* sp.	3 other spp.*	4 other spp.†
At 7 days	− − −	− − −	+ − −	− − −	+ − −	− − −	+ − −	+ + −	+ + +
At 10 ,,	− − −	+ + −	+ + −	+ + −	+ + −	+ + +	+ + +	+ + +	+ + +
At 15 ,,	+ + −	+ + −	+ + −	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

* *Fusarium vasinfectum, Aspergillus flavipes, Helminthosporium* sp.
† *Penicillium terrestre, Penicillium* sp., *Trichoderma viride, Fusarium oxysporum.*

Another method* was followed in the next experiment. On the surface of plates containing a 10 c.c. layer of water agar was placed a sterile filter paper saturated with a sterile nutrient solution of the following per litre composition:

KNO_3 1·0 g., $CaCl_2$ 0·5 g.
K_2HPO_4 0·5 g., $MgSO_4.7H_2O$ 0·2 g.

The plates were inoculated with mycelial strands of the test fungi, care being taken that no agar was transferred with the inoculum. Not one of the eleven isolates of *Rhizoctonia* was able to grow over the filter paper, although the eight other species tested grew well. In the experiment with the McBeth-Sanborn method, however, *Rhizoctonia* produced the blue reaction characteristic of cellulose decomposition, albeit more slowly and less strongly than the typical cellulose-decomposers. One must conclude, therefore, either that the precipitated cellulose was more available to *Rhizoctonia* than that of the filter paper, or that the organism was unable to grow on a cellulose-minerals medium in the absence of agar. Both experiments demonstrate, however, the disadvantage under which *Rhizoctonia* must compete with typical cellulose-decomposers in a soil to which grass or straw meal has been added.

The greatest increase in growth in the soil with grass or straw meal was produced by calcium nitrate, which was evidently more suitable for *Rhizoctonia* in this experiment than was ammonium phosphate or sulphate. Addition of nitrogen even as calcium nitrate, however, failed to counteract completely the depressing effect of the organic supplements upon growth.

In a further experiment of the same type, applications of nitrogen at the same rate were also made to a parallel series of soil without organic supplements (Table 15). Essentially similar results were obtained in this second experiment by the addition of nitrogen

TABLE 14. *Effect of additional nitrogen upon growth of* Rhizoctonia *(in mm.) in soil with organic supplements*

	Soil alone.	Soil + 1 % grass meal				Soil + 1 % straw meal			
	No N	No N	$Ca(NO_3)_2$	$NH_4H_2PO_4$	$(NH_4)_2SO_4$	No N	$Ca(NO_3)_2$	$NH_4H_2PO_4$	$(NH_4)_2SO_4$
After 6 days	29	7	20	16	13	8	22	18	13
After 12 ,,	43	8	30	24	14	9	32	22	13

S.E. after 6 days ±1·4; after 12 days ±1·6.

* Suggested by Dr A. G. Norman, of Iowa State College, U.S.A.

† Kindly determined by Dr J. C. Woodward, Department of Agriculture, Ottawa.

to the soil + grass-meal mixtures; in no instance did the addition of nitrogen to the soil without organic supplement increase growth, and in some series it slightly reduced it.

(v) *Mitigation of the depressing effect of organic matter by reduction in carbon-dioxide content of the soil atmosphere.* The cellulose-decomposing micro-organisms may compete with *Rhizoctonia* not only for essential supplies of soil nutrients, such as nitrogen, but also for the soil oxygen; moreover, the growth of *Rhizoctonia* may be depressed by the output of respiratory carbon dioxide from the cellulose-decomposers. The beneficial effect of forced soil aeration upon the growth of *Rhizoctonia* through untreated soil has been described above (Table 2); the same experiment was repeated with soils to which 1% of grass or straw meal had been added, and growth of the fungus was more than doubled by the forced aeration. Before reporting experiments more precisely designed to reduce the carbon-dioxide content of the soil air in the soils with organic supplements, the effect of carbon dioxide upon the growth of *Rhizoctonia* on potato-dextrose agar may first be described.

TABLE 15. *Effect of additional nitrogen upon growth of* Rhizoctonia *(in mm.) in soil with and without grass meal*

	Soil alone					Soil + 1% grass meal				
	No N	$Ca(NO_3)_2$	KNO_3	$NaNO_3$	NH_4NO_3	No N	$Ca(NO_3)_2$	KNO_3	$NaNO_3$	NH_4NO_3
After 6 days	28	26	27	29	24	9	17	17	22	14
After 12 „	43	36	38	41	34	9	28	29	35	28

S.E. after 6 days ±1·5; after 12 days ±1·9.

An experiment was carried out in two steel cylinders; the required concentration of carbon dioxide was established in one cylinder, whilst the other cylinder contained air with carbon dioxide at normal atmospheric concentration, to serve as a control. Five concentrations of carbon dioxide, from 1 to 20%, were studied, in as many separate experiments. The required concentration of carbon dioxide was secured by passing the calculated volume of the gas into the cylinder under pressure through an inlet valve at the top, whilst a corresponding volume of displaced air was permitted to escape through an outlet valve at the bottom of the cylinder. The composition of the atmosphere in the cylinder was checked at the beginning and again at the end of the experimental period with Fisher's modification of the Haldane gas apparatus (Matuszak, 1934). Twelve plates of potato-dextrose agar inoculated with isolate R_1 were placed in each cylinder, upside down with lids off in a removable frame; colony diameters were measured after 5 days, and the results of the five separate experiments are given together in Table 16.

Experiments demonstrating the effect of reducing the carbon-dioxide content of the soil atmosphere may now be described. The first experiment was carried out in large glazed clay crocks. At the bottom of each crock was placed an open dish filled with a saturated solution of alkali; this was covered with wire gauze and then with a 1 in. layer of cotton-wool. The crock was filled to within 2 in. of the top with Red River soil (with or without 1% straw meal), previously adjusted to a moisture content of 40% saturation, whilst at the same time six glass slides were placed in position and inoculated with isolate R_1. The surface of the soil was covered with another 1 in. layer of cotton-wool, on top of which was placed a second dish filled with a saturated solution of alkali. The top of the crock was covered with a double layer of cellophane, tightly stretched and secured. Three different alkalies were used in different series; two crocks were allotted to each series, so that measurements were obtained from twelve slides per series. The *p*H value of the soils, determined at the beginning and end of the experimental period of 7 days, did not vary from *p*H 7·0 in any series (Table 17).

TABLE 16. *Diameter of plate colonies of* Rhizoctonia *(in cm.) in atmospheres of different carbon-dioxide content*

% CO_2 in atmosphere ...	1	2·5	5	10	20
Growth in air + CO_2	7·5	6·7	4·9	4·5	3·1
Growth in air alone	7·8	7·8	7·9	8·2	8·2

S.E. ±0·21.

TABLE 17. *Effect of reducing carbon-dioxide content of soil atmosphere upon growth of* Rhizoctonia *(in mm.) in soil with straw meal*

	Soil alone	Soil + 1% straw meal			
Alkali in dishes	None	None	NaOH	KOH	$Ba(OH)_2$
	36	18	32	31	32

S.E. ±1·7

The presence of the alkali improved growth in the soil + straw-meal series until it almost equalled that in the untreated soil (the observed differences are not actually significant).

A second experiment was suggested by the hypothesis of Garrett (1936) that alkaline soils act as carbon-dioxide acceptors, and by absorbing respiratory carbon dioxide accelerate the growth of *Ophiobolus graminis* along the plant roots. The growth of *Rhizoctonia* was accordingly compared in a series of soils adjusted to different reactions, with and without grass or straw meal. Batches of Red River soil were treated with various amounts of sulphuric acid and calcium oxide, respectively, brought to a moisture content of 40% saturation, and incubated for 3 weeks at 30° C. The soils were then dried, crushed and sieved; *p*H values were determined after remoistening the soils, and again at the end of the experimental period. Four-inch unglazed clay pots were used as soil containers; two pots each containing three slides (inoculated with isolate R_1) were allotted to each soil series. The soil-moisture content was adjusted to 40% saturation for this experiment; growth was measured after 12 days (Table 18).

In the soil without organic supplement a *p*H value of 7·0 was optimum for growth of *Rhizoctonia*, as in a previous experiment (Table 4): where either grass or straw meal had been added to the soil the optimum *p*H value for growth rose to 8·0. This result agrees with the hypothesis that accumulation of respiratory carbon dioxide is one of the factors limiting growth of *Rhizoctonia* in soil treated with grass or straw meal.

TABLE 18. *Effect of soil reaction upon growth of* Rhizoctonia (*in mm.*) *in soil with and without organic supplements*

	$+H_2SO_4$			No treatment	+CaO					
	1·3%	1·0%	0·8%		0·6%	0·9%	1·4%	3·2%	5·0%	6·0%
Mean soil *p*H value ...	5·5	6·3	6·6	7·0	7·3	7·5	7·6	8·0	8·2	8·5
In soil alone	19	24	27	44	38	35	30	28	27	21
In soil+grass meal	11	12	12	13	21	23	25	27	25	17
In soil+straw meal	9	10	14	13	19	22	25	27	26	18

S.E. ±1·1.

The writer wishes to express his deep appreciation to Mr S. D. Garrett, under whose direction the work was conducted.

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Experiments to determine the effect of certain wood preservatives on the growth and cropping of the cultivated mushroom (*Psalliota campestris*)

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A small-scale laboratory method of determining the effect of wood preservatives on growth and cropping of the cultivated mushroom is outlined. Experiments with a number of well-known wood preservatives are described. According to these experiments wood treated with the following preservatives had no adverse effect on mycelial growth or on croppings: 5 % copper sulphate solution, green Cuprinol, 5 % Celcure solution, 2 % Triolith (Wolman salts) solution. On the other hand, wood treated with 2 % Chromel salt and coal-tar creosote reduced the vigour of growth of mushroom mycelium to a depth of ½ in. in compost that was in contact with it. Under these conditions, however, the mycelium was not killed nor was the cropping reduced even when the treated wood was only 1½ in. below the casing soil. As in all experiments the treated wood was covered with compost, the chance of vapours from the preservatives coming into contact with sporophores was very small, and the possibility of harmful effect in mushroom houses from such vapours should not be ignored.

The experiments indicate that full-scale trials in commercial mushroom houses could be undertaken with copper sulphate, green Cuprinol, Celcure and Triolith with negligible risk to the crop, but that it is advisable to carry out trials on a smaller scale with Chromel and coal-tar creosote of known composition to determine whether these preservatives have any adverse effect on cropping. Owing to the great range in composition shown by coal-tar creosote it is most important that the conclusions reached with the particular sample used in the above experiments should not be applied to creosotes in general.

Introduction

Wood preservatives and disinfectants are widely used in mushroom houses to preserve timber from wood-rotting fungi or to destroy fungal and insect pests of mushrooms. It might be expected that these materials or any volatile constituents in them would be harmful to mushrooms and occasionally damage to crops is reported, but in general the experience of growers suggests that they are safe to use. There is little experimental evidence regarding the suitability of many of the materials in use, but copper sulphate and coal-tar creosote are referred to in the literature. These materials are used to preserve wood and also to disinfect bed-boards between cropping periods. The boards are usually dismantled, scrubbed as clean as possible and left to soak overnight in a tank of the disinfectant: on removal from the tank they are commonly given only a short exposure outside before being reassembled in the houses. Referring to experience in the U.S.A., Beach (1937) remarks: 'Considering low cost, fungicidal value, penetration, permanence, and preservative action on wood, 1 to 2 per cent copper sulphate solution is one of the best. This solution does not affect mushroom growth in any visible way when applied to boards as has been shown by experimental tests.' Lambert (1932) advises against the treatment of bed-boards with creosote because in preliminary trials with boards that had been pressure treated with this material, the growth of spawn was found to be retarded, especially when the boards had been freshly treated. On the other hand, Read (1941), in this country, carried out experiments with several samples of creosote and concluded that creosote fumes are injurious to mushrooms if present in sufficient concentration, but that in normal practice most of the volatile fractions of creosote will have disappeared from woodwork before beds are spawned and damage to the crop would be unlikely to occur. The present writers have visited mushroom farms where bed-boards are dipped in creosote and reassembled before they are properly dry, and a strong smell of creosote fumes is noticeable in the houses throughout cropping without any adverse effect showing. Wood (1939) remarks that from the commercial grower's point of view the use of creosote offers the most successful and convenient method of getting rid of *Fusarium*. The conflicting evidence regarding creosote is probably due to variation in its chemical composition. Samples differ as shown by distillation range and content of tar acids, and it seems reasonable to assume that the smaller the percentage of the more volatile fractions and the lower the content of tar acids, the less likely is damage to mushrooms to occur.

In view of the uncertainty regarding the suitability of nearly all the common wood preservatives for use on the timber of mushroom houses, particularly the bed-boards, it was decided to carry out the following small-scale experiments in which the mushroom mycelium was brought into very close contact with wood containing a number of selected wood preservatives.

MATERIALS USED

The wood preservatives selected were as follows:

A. 5% copper sulphate solution.
B. Cuprinol, green grade.
C. 5% Celcure solution.
D. 2% Chromel solution.
E. 2% Triolith (Wolman salts) solution.
F. Coal-tar creosote to a British Standard specification, type A.

The composition of a number of these preservatives cannot be given fully as some are proprietary preparations of which only a brief description is available.

Cuprinol contains metallic salts of high molecular weight in an organic solvent.

Celcure contains dichromates, copper sulphate and chromium acetate.

Chromel contains mercuric chloride, potassium dichromate, sodium nitrite and caustic soda.

Triolith is described as a fluoride-phenol-chrome mixture.

The *creosote* used was a normal creosote having the following analysis:

Specific gravity	1·059	
Distillation up to 205° C.	3·75	% by vol.
Distillation up to 230° C.	25·25	,,
Distillation up to 315° C.	69·25	,,
Tar acids	9·00	,,

It thus consisted largely of compounds of very low volatility.

The timber used consisted of disks of Scots pine sapwood, $2\frac{3}{4}$ in. diam. by $\frac{1}{4}$ in. thick, which were vacuum impregnated with the above preservatives at the Forest Products Research Laboratory. After treatment the disks were left in the open for about a month to allow some of the salts to become 'fixed' in the wood and the more volatile constituents of the creosote to evaporate.

For reasons explained later, a number of disks were drilled after treatment with $\frac{1}{8}$ in. holes in two concentric circles $\frac{5}{16}$ in. and $\frac{12}{16}$ in. respectively from the centre of the disk, eight holes in the outer circle and three in the inner circle.

The average weights of drilled and undrilled disks, together with the average absorption of preservative by the disks are given below:

Ref. letter	Preservative	Wt. of solution absorbed Av. of 6 g.	Wt. of undrilled disks Av. of 2 g.	Wt. of drilled disks Av. of 2 g.
A	Copper sulphate	15·7	10·13	9·98
B	Green Cuprinol	3·95	12·33	10·96
C	Celcure	16·41	10·58	11·29
D	Chromel	14·18	12·60	12·81
E	Triolith	14·75	12·86	11·09
F	Creosote	3·31	12·59	13·22
G	Untreated	—	12·16	9·26

Impregnation by pressure treatment is the method recommended by the makers for all substances except Cuprinol, for which external application—said to be equally effective—is advised. The weights of the disks are affected by slight variations in thickness which accounts for some of the drilled ones being heavier than the comparable undrilled disks.

EXPERIMENTAL METHODS

Two series of experiments were run concurrently. In the first the object was to imitate the conditions in mushroom houses where bed-boards had been treated with preservative, and to note what effects the preservative had on mycelial growth and cropping. In the other the object was to force the mycelium at an early stage of growth into close contact with timber treated with preservative and observe how growth and cropping were affected.

A laboratory method of growing mushrooms in beakers was used that had previously been found successful by one of the writers (N. H. P.) in another investigation on casing soils. The growing medium consisted of fresh, moist, pure-culture spawn (Wood, 1937) or sterilized horse-manure compost in very short condition, such as is used for the culture of spawn in bottles. The advantages of the method are that variability in the compost and in the casing soil are reduced to a minimum, the beakers can be kept under uniform conditions and stages in growth can readily be observed. A rough estimate of yield can also be obtained from the number and rate of appearance of sporophores.

In the first series of experiments, a disk of wood, containing one of the above preservatives, was placed in the bottom of a 600 ml. tall form, lipless Pyrex beaker of 3 in. internal diameter and covered with horse-manure compost, already sterilized and in short condition, which was pressed down firmly to a depth of 2 in. On top of this was placed, in similar fashion, a 1 in. layer of freshly made pure culture spawn grown on horse-manure compost in a very short state. The spawn was broken up thoroughly with the hands and sufficient mixed together for all experiments. Spawn of a white variety was used, as this is said to be less vigorous than cream or brown varieties. On top of the spawn was placed a 1 in. layer of uniformly moist soil which was known to promote the production of sporophores. In this arrangement the relative thickness of compost plus spawn to wood was 8 to 1 as against 6, 7 and 8 to 1 in commercial beds, but the casing soil was only 3 in. from the treated wood as against 6–8 in. in mushroom beds. Unlike the timber of bed-boards the underside of the disk was not exposed to the air. On the whole, these conditions were probably more severe on mushroom growth than in commercial practice.

In the second series of experiments a $1\frac{1}{2}$ in. layer of horse-manure compost was put into the bottom

of the beaker, a disk drilled with holes was placed on top and covered with another $\frac{1}{2}$ in. of compost, and then the spawn and casing soil added as before. As far as possible the disks were placed centrally in the beakers, leaving an annular space $\frac{1}{8}$ in. between the edge of the disk and the side of the beaker. In this arrangement the mycelium, in order to get past the disk, would have to pass within $\frac{1}{8}$ in. of treated wood if the disk remained in a central position and within $\frac{1}{4}$ in. if it became displaced, and if it grew through the holes in the disk it would have to pass within $\frac{1}{16}$ in. of treated wood. The arrangement thus provided a drastic test of the effect of preservatives on the growth and cropping of the cultivated mushroom.

In addition to the above, beakers containing disks of untreated wood (ref. letter G) and others without wood (controls) were included. All treatments and controls were tested in duplicate, making a total of thirty beakers.

The beakers were set up on 27 July 1941 and placed on a bench in a room where the temperature fluctuated from about 62 to 65° F. during any period of 24 hr. The beakers were randomized in four rows or blocks, each row consisting of a complete series and alternate rows being of the same series. Observations were made daily and the soil moistened when necessary with distilled water.

Observations on growth of mycelium

Series I. The mycelium reached the bottom of the beakers and made contact with the disks within 4 days except in the beakers containing disks of wood treated with 2% Chromel solution and coal-tar creosote, treatments D and F respectively, where the mycelium was arrested in growth $\frac{1}{2}$ in. above the disk. In the following days growth became denser in all beakers, but only extremely fine, sparsely distributed hyphae appeared in the $\frac{1}{2}$ in. layer immediately above disks D and F. Good agreement between duplicates was obtained.

Series II. The mycelium passed the disks and reached the bottom of the compost within 4 days in the beakers containing disks of wood treated with 5% copper sulphate solution and Cuprinol, treatments A and B respectively. In all other beakers, including those containing untreated wood, there appeared to be a check to growth just above the disks. However, on the seventh day the disks had been passed and growth was uniformly strong throughout the compost except in the beakers containing disks D and F where the hyphae were extremely fine and thinly scattered from the level of the spawn to $\frac{1}{2}$ in. below the disk, but lower down were as strong and as dense in growth as in other beakers. No subsequent change in growth was observed and good agreement between duplicates was obtained.

The disks were removed on 3 Dec., 127 days after the experiments were set up. In a number of instances there were no signs of mycelium in the zone of compost next the disk and the compost was not altered in appearance to any appreciable extent. After such a long time, however, the mycelium might tend naturally to disappear, particularly as the compost in this zone was wetter than elsewhere. Moreover, the growth below the disks in ser. II is convincing evidence that the mycelium had passed through compost in very close contact with the disks.

Examination of the disks in ser. I showed thick strands of mycelium over the untreated disks and the disks treated with Triolith. In ser. II, thick strands of mycelium radiated from the holes across the surfaces of the untreated disks, the disks treated with copper sulphate and the disks treated with Celcure. In other instances there was no visible growth of the mycelium over the disks or through the holes in them.

Observations on production of sporophores

All treatments in both series produced sporophores, a fact which may be taken as evidence that conditions for growth of the mushrooms were by no means unfavourable. The first ones were picked when about 1 in. across, 23 days after the experiments were started and picking continued until 13 Sept. The crop from each beaker was not weighed, as previous experience had shown that the cropping area is too small for reliable differences in yield to be obtained. There were differences in numbers and sizes of sporophores, but nothing sufficiently great to give the impression that any one treatment favoured or inhibited cropping. The lack of definite evidence of any adverse effect on cropping is particularly interesting in the case of treatments D and F, since the sporophores were apparently able to obtain adequate nutriment through the very attenuated mycelium in the top 1 in. of compost, unless of course cropping was sustained in all instances by the 1 in. layer of spawn.

Condition of disks

None of the treated disks showed any appreciable decay at the end of the experiment, and in only two of the untreated samples was slight superficial decay present. Probably the conditions were too moist to permit any serious decay of the wood to develop during the period of test.

The writers express their thanks to the Forest Products Research Laboratory, Princes Risborough, Aylesbury, Bucks, for much valuable help. The disks of wood used were specially prepared and impregnated with preservatives at that laboratory and were examined there for signs of decay when the experiments were completed.

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Value of phloem necrosis in the diagnosis of potato leaf-roll

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(With Plate 2)

In plants infected with leaf-roll virus a type of phloem obliteration and necrosis occurs which is distinct from any abnormality produced by other pathogens or arising from physiological causes. The necrosis occurs in the primary phloem only of the bicollateral bundles. The affected tissue reacts with phloroglucinol in HCl. It was present in all of 179 plants of 33 varieties showing secondary leaf-roll which were examined and was not found in any of 83 healthy plants of 20 varieties. The amount of necrosis varies in different plants. If the disease is severe, necrosis may extend to almost all parts of the plant except the stolon, tubers and roots. If the infection is mild it may be confined to a very few strands in two or three nodes near the base of the main stem. Phloem necrosis can always be found before leaf rolling is apparent.

In primary leaf-roll, slight necrosis can be found in the stem near the bases of the lowest rolled leaves and sometimes in the petioles.

A technique is suggested for the use of this symptom in diagnosis.

Introduction

Of the virus diseases causing degeneration of potato stocks in England leaf-roll is the most important. Its diagnosis in the field, however, is often by no means easy, for the symptoms of infected plants, even in one variety, vary considerably, and rolling of the leaves can also result from other causes. Nearly thirty years ago Quanjer (1913) and Quanjer *et al.* (1916) found that some of the primary phloem of leaf-roll plants was necrotic, and claimed that this symptom was sufficiently constant to be of diagnostic value. Since then various workers have studied this phenomenon, but there has been no general agreement. Some agreed with Quanjer, whilst others could not always find phloem necrosis in leaf-roll plants and others held that a similar necrosis occurs in plants not infected with the leaf-roll virus. The work was reviewed by Esmarch (1932) and Esau (1938). Because of the importance of accurate diagnosis of true leaf-roll in potato-seed certification schemes, and that the possibility that phloem necrosis might be of some value as a diagnostic aid in doubtful cases, the occurrence of this feature has been examined in some detail.

Material and methods

The study was concerned mainly with phloem necrosis in plants with secondary leaf-roll, but some newly infected plants were examined. Plants showing all degrees of rolling, pallor and stunting, were examined as well as healthy plants. In some varieties (e.g. Gladstone) the leaves may roll although the plants are apparently free from any infection: a study was made of a few of these. Plants whose leaves were rolling due to injury, to known physiological causes or to infection with other pathogens, were also examined. Attention was paid also to potatoes infected with other viruses.

Some of the plants infected with secondary leaf-roll were examined early in the season to determine the time of onset of phloem necrosis, but the majority of those examined from field crops were taken late in June or in July when most of the crop was about 18 in. above soil level (or about 2 ft. above the tuber). In some cases over- and underground parts of the plant were sectioned, but usually only the nodes, internodes and petioles.

In 1941, all the material used came from the Ministry of Agriculture's potato collections at the

Midland Agricultural College, Sutton Bonington. It was fixed in 1% chrome-acetic acid, embedded in paraffin wax and sectioned at 10 μ by a rotary microtome. Anatomical stains such as safranin with light green or Delafield's haematoxylin or Pianese III B were used. This technique is not well suited to the material, and in 1942 only hand sections were used. The material was sectioned either fresh or after storage in 50% alcohol or in formalin-acetic-acid alcohol.* Sections were examined unstained or after treatment with phloroglucinol.

Early in 1942, healthy and leaf-roll infected plants of several varieties selected by F. C. Bawden and P. H. Gregory in previous years from fields in several parts of England were grown under glass at Rothamsted Experimental Station. Other infected tubers obtained from K. M. Smith of Cambridge and G. Cockerham of Edinburgh were grown either under glass or outside. Material was taken from various experimental plots at Rothamsted farm and from plots at the Ministry of Agriculture's Plant Pathological Laboratory at Harpenden, also from farms in Kent, Berkshire, Hampshire and Devon. Healthy and infected plants of a number of varieties were examined at Sutton Bonington. Whenever possible each variety was obtained from more than one source, but the results obtained were independent of the source or conditions of growth of the material used.

Anatomy of healthy stem and petiole

The mature healthy stem is usually triangular in cross-section. On the outside is a cuticularized epidermis and immediately within this a layer of collenchymatous tissue several cells wide. The inner cortex and the pith both consist of thin-walled parenchymatous tissue. A few cells of this ground tissue are often completely filled with calcium oxalate in the form of a crystalline sand. The vascular system is bicollateral. The xylem consists usually of three large groups, one at each corner of the stem, connected together by a narrower band. The xylem tissue of the normal stem usually presents a regular surface on the outside but is irregular towards the centre because of the primary xylem groups. Immediately outside the secondary xylem is a smooth narrow band of small, thin-walled, meristematic cells, the cambium. Outside this is the secondary phloem which forms a complete ring usually widening at the corners of the stem. Scattered in the cortex, and in the pith, are many groups of primary phloem tissue. Each group consists of small sieve tubes, smaller companion cells and a few parenchymatous cells (Pl. 1, fig. 1). A whole group is usually about the same size as a single one of the parenchymatous cells surrounding it. One or two fibres may be associated with a group. It is unusual for these to become lignified until very late in the season. In cross-section these groups appear to be isolated from each other, but longitudinal sections of the stem show that the strands anastomose. Such normal stems are figured by Artschwager (1923), and details of the sieve tubes are shown by Crafts (1933).

In the maturing stem certain of the cells of the primary phloem may become obliterated. The walls of these cells are thickened and the lumina may be crushed. They are colourless when fresh and do not react with phloroglucinol or stain with dyes such as safranin. This is probably the 'necrobiose' described by v. Brehmer (1923) and accords with the results of Crafts (1933). He found that after a time sieve-tube nuclei disintegrate and the cells lose their semi-permeable property. He considers that they have now reached their maximum function. Later they become functionless and may be crushed and obliterated.

The petiole differs from the stem in being crescent-shaped in transverse section. The vascular system does not form a closed ring but consists of about six laterally elongated groups arranged in an arc roughly parallel to the lower epidermis of the petiole. Each group consists of xylem with internal and external phloem strands.

Anatomy of plants with secondary leaf-roll

In all the plants examined which were infected with leaf-roll virus only, abnormalities were confined to the vascular system.

Xylem. The primary xylem is normal but the secondary xylem is often differentiated from the cambium irregularly. The rate of differentiation is evidently different on different radii so that the outer edge of the xylem forms a jagged line. The cells are normal in appearance (Pl. 1, fig. 3).

Cambium. The cells are normal in appearance, but instead of the usual smooth ring of tissue, a wavy band follows the jagged edge of the secondary xylem (Pl. 1, fig. 3).

Phloem. The secondary phloem may be unevenly differentiated like the xylem but the cells are apparently normal (Pl. 1, fig. 3).

It is in some of the *primary phloem* strands that marked abnormalities appear. Some or all of the cells of a group may be affected. The walls of the affected cells swell and become pressed together. The lumina are reduced until very narrow or may be completely obliterated. Usually the whole group decreases in size: this is compensated by an increase in size of the surrounding parenchymatous cells which may become distorted and appear to radiate from the necrosed phloem group (Pl. 1, fig. 2). No cells other than those of the primary phloem become necrotic. If seen unstained, the affected cells are a yellowish brown colour. They stain with those dyes which are commonly used to show lignified tissue such as safranin or malachite green (in Pianese III B),

* F.A.A. preserves tissues rather better than alcohol but it is necessary to wash sections for 5 min. in 50% alcohol before a reaction can be obtained with phloroglucinol.

and they also react with phloroglucinol. Because of these reactions, many authors have assumed that the tissue becomes lignified, but these stains are not specific for lignin and a red colour with phloroglucinol in HCl merely shows the presence of a pentose. No serious attempt has been made to identify the substance produced in these necrotic cells, but it has been suggested that it is lignin (Quanjer *et al.* 1916; Esmarch, 1932), suberin (v. Brehmer & Rochlin, 1931) or a wound gum (Esau, 1938).

Distribution and extent of phloem necrosis

When infected tubers are grown the phloem may show effects when the shoots are 5–6 in. long, but necrosis is usually first found when the shoot is about 9 in. Even when not earthed-up, the stem of the potato usually increases suddenly in width a few inches above the tuber. Necrosis is most often first seen at the level of this increase, and throughout the life of the plant the amount of necrosis is at its maximum very near this level. As the plant grows the number of affected strands increases and necrosis spreads both up and down the stem. In any one variety the amount of necrosis is correlated with the severity of the external symptoms.

Infected plants do not at first show any external symptoms. Some necrosis can always be found *before* rolling of the leaves is apparent though it may be very slight. In a plant showing slight external symptoms, necrosis may be confined to one or two strands of primary phloem and may not extend vertically for more than two or three internodes. When external symptoms are severe, phloem necrosis may extend throughout the main stem to within 1 in. of the growing point, it may spread into all the branches, throughout the petioles of all but the youngest leaves, to the tip of the midrib and into the larger secondary veins of some older leaves. It was not found in the smallest veins, in the stolon, in the tuber or in adventitious roots.

Necrosis may occur equally in the internal and external primary phloem, or it may be confined to one or may be severe in one and slight in the other (Pl. 1, figs. 3, 4). The number of affected strands is usually greatest at the nodes. The affected strands are often not evenly distributed around the stem: frequently, but not invariably, the majority are found in those bundles which pass into the leaf traces.

It will be seen that the extensiveness of the internal symptom is as variable as that of the external. However, as necrosis always occurs before leaf rolling and as it always commences near the base of the main stem, it will always be found here in a suspected plant which is affected, however slightly, by this virus.

Varieties examined

Table 1 is a list of varieties of which either healthy plants or plants with secondary leaf-roll were examined. In the column 'Known to be infected', the virus has been transmitted either by grafting or insects. The plants in the next column had shown symptoms of secondary leaf-roll for at least two successive generations. Those in the last column were selected from field crops in 1942.

TABLE 1. *Potato varieties examined*

	Nos. of plants examined			
Variety	Healthy	Known to be infected	Showed symptoms in two successive years	Field diagnosis
Ally	3	—	—	1
Alness	2	4	6	2
Arran Banner	14	3	—	3
Arran Chief	1	1	—	—
Arran Comrade	—	1	—	2
Arran Consul	1	1	—	2
Arran Pilot	1	1	—	—
Arran Victory	—	2	—	—
Bishop	—	1	—	—
British Queen	5	7	1	—
Craig's Defiance	2	1	1	—
Di Vernon	1*	2	—	1
Doon Early	1	1	—	—
Doon Eire	—	—	—	1
Doon Star	4	4	6	11
Duke of York	1	1	—	—
Dunbar Cavalier	—	—	—	1
Dunbar Rover	—	1	—	—
Dunbar Standard	—	1	—	1
Eclipse	—	—	—	1
Epicure	—	1	—	1
Gladstone	3+5*	2	—	2
Golden Wonder	1	1	—	2
Great Scot	1	2	—	1
Katahdin	1	—	—	—
Kerr's Pink	—	1	—	1
King Edward	5	5	4	9
Majestic	28	3	6	42
May Queen	—	1	—	1
President	2	9	—	—
Redskin	—	1	—	1
Royal Kidney	—	—	—	1
Sharpe's Express	1	—	—	—
Up-to-Date	—	7	1	1
U.S.D.A. Seedling No. 41956	—	1	—	—

* Indicates the number of healthy plants showing varietal rolling.

In every one of the 179 infected plants of the thirty-three varieties examined, some phloem necrosis was found. Usually the number of plants examined in any one variety was insufficient to indicate the existence of varietal differences. If these occur, they must obviously be in the extent of the necrosis. Fairly large numbers of Majestic and King Edward plants were examined and the latter seemed generally to show more necrosis, about three-quarters of the primary phloem often being destroyed in the stem near ground level. In Majestic it is more

usual to find necrosis in about half the primary phloem strands at this level, but in a mildly diseased plant only a very few strands are necrotic. As the external symptoms on the King Edward plants examined were very marked, this apparent varietal difference is probably only a reflexion of the relative intensity of the virus attack.

Eighty-three healthy plants of twenty varieties were examined. Usually these were taken as controls to the infected plants, but a number (Majestic and Arran Banner) were examined during September when the tops were dying off. This was to test the possibility of phloem necrosis resulting from senescence as suggested by Esmarch (1919) and by Schander & Bielert (1928). Until very late in the season the only abnormality seen in healthy plants was the obliteration previously referred to which was easily distinguished from that due to leaf-roll virus by lack of discoloration and by staining properties. In old plants degeneration of the tissues commences in the region of the primary xylem and internal phloem. Occasionally cells were found in the ground tissue or in the phloem whose contents were discoloured and stained red with phloroglucinol. Such cells were occasionally crushed but the walls were not thickened and usually remained unstained. Occasionally cells were found the corners only of whose walls took a phloroglucinol stain. Such abnormalities were rare, could not easily be mistaken for the phloem necrosis due to leaf-roll virus, and were not found until much later than any attempt to diagnose secondary leaf-roll would normally be made.

It seems probable that where phloem necrosis has been described in apparently healthy plants sufficient care has not been taken to test for the presence of a latent virus infection. During the course of these experiments, a Doon Star plant was selected from the field as showing marked symptoms of leaf-roll. A plant of the first generation grown in the glasshouse showed no external symptoms of leaf-roll although phloem necrosis occurred. Some plants of a further generation were grown under glass and others in the open. All showed phloem necrosis and external symptoms of leaf-roll virus.

Primary leaf-roll

The newly infected plants examined comprised eight Majestic infected by grafting of an Up-to-Date infected scion, three Majestic infected in the glasshouse by *Myzus persicae* and three Alness infected in the field.

When the virus was transmitted experimentally by grafting, and primary leaf-roll symptoms had developed in the side branches, necrosis first appeared near the base of the branch and extended upwards.

When it was transmitted by insects, some slight necrosis was usually found in the main stem about the level of insertion of the lowest rolled leaves. As the upper leaves unfolded and the stem elongated, necrosis extended slowly upwards. As the plants examined were few and all were infected relatively late in the season, it was not determined whether necrosis also extended downwards. Very slight necrosis was sometimes found in the petioles of the lowest rolled leaves.

It was not determined whether, as in secondary leaf-roll, the necrosis is present before rolling occurs.

Comparison with some other abnormal plants

A number of potatoes which were not infected with virus but whose leaves were rolling from other causes were examined.

In some varieties a cupping of the leaves starting from the base frequently occurs. The cause is unknown, but the cupping is very similar to that induced by leaf-roll virus. No other symptoms such as stunting, pallor or changed habit accompany it. This type of rolling is most prevalent in the variety Gladstone and also occurs in Di Vernon. Anatomical examination showed nothing abnormal in the structure of such plants.

Mechanical injury to the stem which prevents the starch assimilated in the leaves from being carried away to the tubers may also cause the leaves to roll. In this case no structural abnormalities could be found. Nor could phloem necrosis be found when a leaf-rolling condition resulted from injury due to *Rhizoctonia*.

Boron deficiency may cause not only rolling of the leaves but also the production of anthocyanin as in severe cases of leaf-roll virus. Van Schreven (1935) found that the phloem as well as the cambium and ground parenchyma may become deformed by extension and compression. Groups of cells may be broken down into dark brown masses. It is not known whether these necrotic tissues react with phloroglucinol. Boron deficiency differs from leaf-roll in that tissues other than the phloem are affected. Necrosis commences at the growing points of the roots, then those of the stem are affected, next the axillary buds and lateral shoots are killed, then the stelar structure of the nodes commencing at the top and finally the internodes are affected. Xylem development is very poor, but the secondary phloem is excessively developed.

'Blackleg' produces symptoms very similar to those of a severe leaf-roll and necrosis does occur. This, however, starts in the xylem and thence spreads to other tissues.

Some other viruses may cause necrosis of certain tissues and should therefore be considered. Necrotic areas appear on the leaves following infection with some strains of *X* virus. Destruction of the tissues in this case commences in the palisade cells and may pass from there to the veins. Artschwager (1923) sometimes found phloem necrosis in stem streak but more characteristic of this disease are changes in the collenchyma which spread thence to other tissues. With leaf-drop streak the author finds, as Quanjer

(1931) and Bawden (1932) described, necrosis starts in the collenchyma and passes from there to other tissues. Quanjer (1931) and Bawden (1932) agree that in top necrosis the phloem is abnormal. Necrosis commences in the internal primary strands, but it rapidly radiates from these across the surrounding tissues.

Examples of diseases similar in one or other of their symptoms to those of leaf-roll virus could be multiplied almost indefinitely. However, no convincing statement has been made that any other pathogen or any physiological condition causes a necrosis confined entirely to the primary phloem strands.

Diagnostic value of phloem necrosis

It is beyond the scope of this paper to describe the structure and ontogeny of the various phloem constituents of the normal plant. It has been under consideration for more than a century and the most recent review of the work is that of Esau (1939). Sufficient has been said here to show that although changes occur in the phloem of the normal potato which are in some aspects similar to those induced by leaf-roll virus, the two types of obliteration are easily distinguished.

The particular form of necrosis described, which is confined entirely to the primary phloem strands, seems to occur only in plants infected with leaf-roll virus. As it was found without difficulty in all the plants of the thirty-three varieties described here as well as in those varieties, including some normally grown in parts of Europe and North America, described from time to time by other workers, it is reasonable to suppose that phloem necrosis always occurs in plants infected with this virus. That some workers have failed to find it may be due to its localization in some cases, especially in mildly diseased plants, to certain parts of the main stem.

As phloem necrosis can be clearly demonstrated by a comparatively simple technique, it should prove of practical value in the diagnosis of doubtful cases of leaf-roll infection.

Suggested technique for the diagnosis of secondary leaf-roll

A piece is taken from near the base of a *main* stem. It should extend about 1 in. below the soil level (i.e. top of earthed-up ridge) and include about six nodes above ground. Leaves and branches are trimmed off. The stem can be sectioned at once, but it is probably more convenient to preserve it to section at leisure. For this, it is cut into pieces of a suitable length to handle when sectioning by hand and the pieces are dropped into a bottle of 50% alcohol. If no alcohol is available, the piece of stem is cut slightly longer (about 1 in. at each end) and kept whole, preferably in an air-tight tin, until it can be sectioned or preserved. (Stems have been kept dry for as long as a week before soaking and sectioning, but if not in a preservative they become difficult to handle after 1 or 2 days.)

Sections are cut by hand through the *nodes* and put into phloroglucinol solution (1% in 50% alcohol) on a slide. After 1 min., the phloroglucinol is drained off and replaced for 1 min. by 50% HCl. This is drained off and the section mounted in water and examined under the microscope at about × 100.

In a healthy plant the xylem will be a purplish red colour, most of the phloem will be colourless, but the fibres may be pink or occasionally red. All other tissues will be colourless. In a leaf-roll infected plant, some of the primary phloem strands will be a yellowish red. Present experience indicates that, if leaf-roll virus is present, phloem necrosis will be found in some of the six nodes which it is suggested should be examined. It is suggested also that, if the variety under consideration is an unfamiliar one, a healthy stem and if possible the stem of a plant undoubtedly infected with leaf-roll virus should also be examined for comparison.

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EXPLANATION OF PLATE 2

All illustrations are from hand sections of stems stained in phloroglucinol. The photomicrographs were taken with a Leitz 'Makam' camera, a mercury vapour lamp screened with a no. 62 Wratten filter providing the illumination.

Fig. 1. Majestic, healthy. The primary phloem group consists of a number of cells considerably smaller than those of the surrounding ground tissue. (×450.)

Fig. 2. King Edward infected with leaf-roll virus. In the centre is a necrotic phloem group. The cells are crushed and distorted so as to be individually indistinguishable. The whole group is shrunken and the cells immediately around it are enlarged and distorted so that they appear to radiate from it. At the lower right-hand corner of the field is part of a normal phloem group. Unstained fibres are seen at the top of the field. (×450.)

Fig. 3. King Edward infected with leaf-roll virus. This section shows the irregular development of the secondary xylem and phloem from the wavy band of cambium cells. Most of the external primary phloem groups are necrotic and consequently heavily stained. (×140.)

Fig. 4. Majestic infected with leaf-roll virus. This section shows a number of internal phloem groups embedded in the pith. Some are normal; others fully necrotic and deeply stained; others are in the process of obliteration and are more faintly stained. (×140.)

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The moisture content of timber in relation to attack by *Lyctus* powder-post beetles

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(With 3 Text-figures)

The relation between moisture content of oak sapwood and rate of development of *Lyctus brunneus* has been investigated at 23° C. Oviposition took place normally in samples of various moisture contents between 5·5 and 23·5 %. The eggs hatched in wood of 7·7 % moisture content, but not of 6·1 % moisture content, although the embryos developed fully. The rate of gain in weight of young larvae increased to a maximum as the moisture content of the wood rose to about 14·2 % and then fell again. The rate of development from egg to adult followed a somewhat similar curve with a maximum at 16 % moisture content. At all moisture contents the first male emerged a few days earlier than the first female. The average weight of the male beetles was less and their moisture content greater than those of the corresponding females. The average weight of the beetles showed a tendency to increase at moisture contents of the wood above 9·7 %. The average moisture content of the males was fairly constant up to 15·6 % moisture content of the wood, but that of the females rose slowly as the moisture content of the samples increased. In wood of 15·6 % moisture content at 23° C. the minimum period of development from egg to adult was 167 days; at 14·2 % moisture content and 27·5° C. this was reduced to 142 days. Green timber was shown to be liable to infestation as soon as a superficial skin was formed of a suitable moisture content; the upper limiting moisture content is probably the fibre saturation point. Control of moisture content of timber cannot be achieved sufficiently easily for it to become an important method for the control of *Lyctus* infestation.

INTRODUCTION

The *Lyctus* powder-post beetles are insects of world-wide economic importance, whose larvae tunnel extensively in the sapwood of many species of commercial hardwood timbers in both temperate and tropical regions. Knowledge of their biology and of methods for their control has increased greatly in recent years, and is summarized in *Forest Products Research Bulletin*, no. 19 (1941).

The wood in which the insects live plays a most important role in their economy, for it is not only the sole source of energy for boring, growth and development, but also acts as a buffer against sudden and

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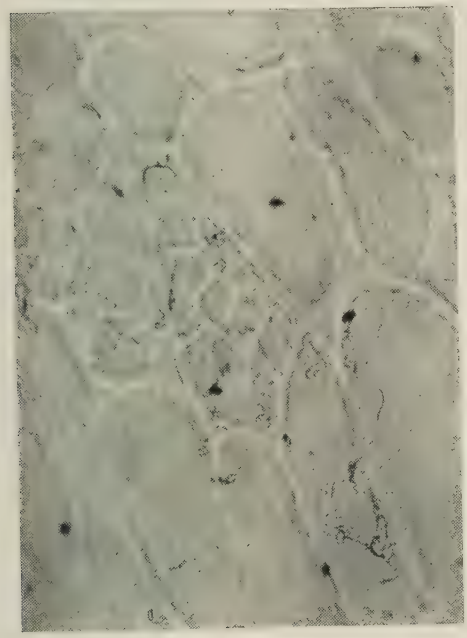

Fig. 1.

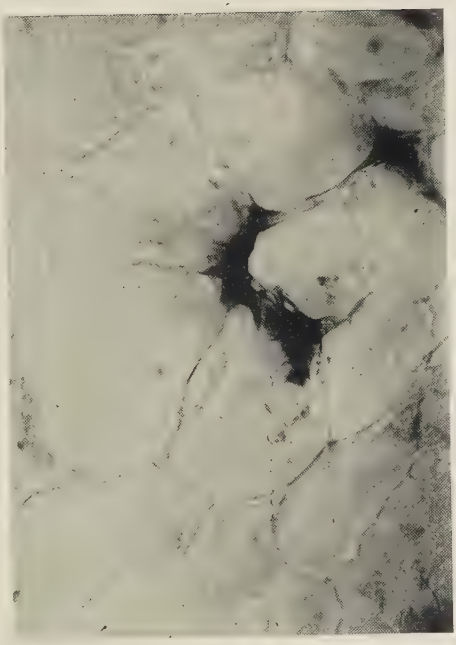

Fig. 2.

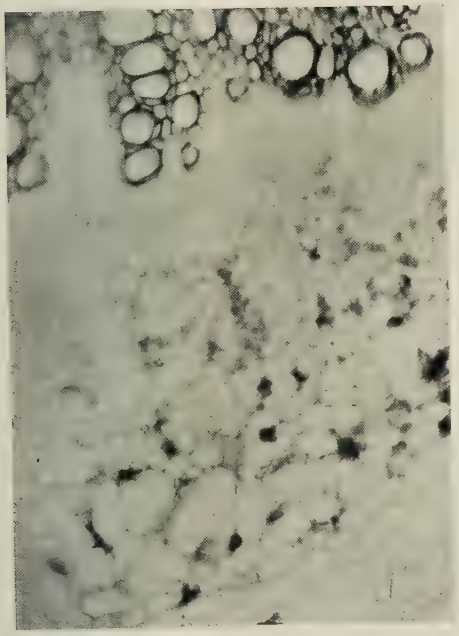

Fig. 3.

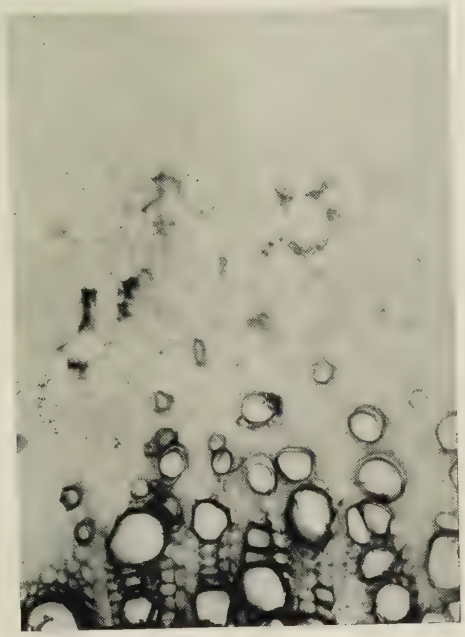

Fig. 4.

F. M. L. SHEFFIELD—VALUE OF PHLOEM NECROSIS IN THE DIAGNOSIS OF POTATO LEAF-ROLL

extensive changes in external physical conditions. The value of this buffering action is evident, when it is remembered that larvae can progress through wood only with extreme slowness and cannot therefore move away quickly from areas in which conditions become adverse. Extremes of humidity, for example, must alter the moisture-content equilibrium of the wood, a slow process, before they can affect larvae living inside a block of wood.

The influence of the moisture content of timber upon the development of *Lyctus* infestation has received relatively little attention, and the object of the present investigation has been to gain more information about the importance of moisture content in relation to attack by powder-post beetles. These insects are generally known as borers in partially or recently seasoned wood, that is to say, they live in wood, the water content of which is below fibre saturation point, about 30%. Green timber is not liable to attack and, when infestation is present in wood with a high mean moisture content, the insects are always confined to a small area in which superficial drying has occurred. For example, *Lyctus* larvae may occasionally be found working in the ends of oak logs, where the sapwood has been exposed by oblique axe cuts through the bark. The moisture content of timber in which *Lyctus* is most frequently found is between 12 and 15%, and conservation of water in such an environment is clearly of importance. Wood particles ingested by larvae are saturated by the digestive juices, yet the faeces in the hinder part of the rectum appear dry. The water must be resorbed through the walls of the rectum, and its removal from the wood is no doubt made more efficient by the close contact of the distal ends of the Malpighian tubules with the hind gut (Parkin, 1936). In the adult stage, when such strict moisture conservation is probably not so necessary, the distal ends of the tubules are no longer bound to the hind gut.

LITERATURE

Although the literature dealing with the biology and control of the *Lyctus* powder-post beetles is considerable, information on the effect of different moisture contents of timber upon the development of infestation by these insects is meagre. Snyder (1916) stated that 'Wood that has been seasoned less than 8 to 10 months will not be attacked by *Lyctus* beetles', a statement which is still being repeated in United States publications, in spite of abundant evidence to the contrary. Fisher (1928) is the only worker who has attempted by laboratory experiment to investigate the effect on the development of infestation of a range of moisture contents of wood. He has reported that oak sapwood, exposed to *Lyctus* within 2–3 weeks of felling, became infested, that larvae of *L. brunneus* developed at moisture contents from 10 to 28% and that no attack occurred below 8%. Beeson & Bhatia (1937) concluded from insectary observations that the larvae would thrive between 10 and 50%, the higher moisture content being the more favourable, and that wood of less than 10% moisture content would not be attacked. They also recorded that the timber of *Bombax malabaricum* was liable to infestation by *L. africanus* a few days after felling and sawing.

EXPERIMENTAL WORK

Three experiments were planned to yield information upon the effect of the moisture content of timber upon oviposition, hatching, early larval development and length of life cycle. A fourth experiment was carried out to determine the length of the life cycle under very favourable conditions of temperature and moisture content. Finally, a special test was undertaken to settle whether *Lyctus* could develop successfully in newly felled timber.

Material

The wood used in the first four experiments was taken from a log of green oak having an exceptional width of sapwood, which enabled samples to be cut $4\frac{1}{2}$ in. long and 3×2 in. in cross-section. As starch is the principal food material of the larvae and beetles avoid oviposition in starch-free wood (Parkin, 1936), care was taken to ensure that the very high initial starch content of this sapwood was maintained during the drying and conditioning period. Apart from the samples already mentioned, which just fitted into desiccators 6 in. diam., a number of small samples $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$ in. were also prepared.

Since the conditioning of samples of wood to a given moisture content can be achieved only with considerable difficulty, it was decided that, for the purposes of this investigation, it would not matter if the moisture contents of the samples failed to agree with the expected values, so long as they formed a series covering a reasonable range. Potassium hydroxide solutions were chosen to humidify the desiccators, because they would prevent accumulation of carbon dioxide and not give off harmful fumes, if bore-dust from the samples should fall into the liquid.

After cutting from the log, the samples were partly air-dried by storage in the laboratory for a fortnight and were then conditioned preliminarily during the following month to 11–12% moisture content in a room maintained at 25° C. and 60% relative humidity. A slat $\frac{1}{10}$ in. thick was then sawn from a 3×2 in. surface of each large block. Each slat was split into two approximately equal pieces, the first of which was dried and its moisture content determined. The second piece was also weighed as soon as cut and its dry weight calculated later from the determined moisture content of the other half.

A large block, two small samples ($\frac{1}{2}$ in. cubes) and a half-slat were placed in the upper part of each of

eight desiccators maintained at 23° C. The desiccators contained in the lower part 200 ml. of aqueous KOH solutions of various strengths calculated from the data collected by Buxton (1931) and Buxton & Mellanby (1934) to give a set of selected relative humidities. These humidities were determined from curves, provided by the Seasoning Section of the Laboratory, to be in approximate equilibrium at 23° C. with oak samples of the desired moisture contents, bearing in mind that some samples would be equilibrating to above and some to below about 11 % moisture content. The slat was weighed at intervals to check the course of moisture-content changes in each desiccator. After 2 months, equilibrium had practically been established and infestation of the samples was started.

Table 1 shows the moisture contents originally aimed at, the estimated relative humidities in equilibrium at 23° C. for oak samples conditioned from 11 % moisture content, and the mean moisture contents of the slats during the periods from the start of infestation of the large blocks until the first emergence of beetles from the corresponding block.

Table 1. *Data relating to the conditioning of samples of oak sapwood to a range of moisture contents at* 23° C.

Moisture content* desired %	Equilibrium relative humidity %	Mean moisture content* obtained %
3·5	10	5·5
5·0	18	6·1
7·0	29	7·7
8·5	36	8·7
10·0	44	9·7
15·0	76	15·6
20·0	86	19·2
25·0	92	23·2

* Water lost at 105° C. expressed as percentage of oven-dry weight.

The relationship between the equilibrium relative humidity and the mean moisture content actually obtained is shown graphically in Fig. 1. The divergence of the moisture contents obtained from those desired, increases as the values become more extreme and is probably explained by the relatively large volume of wood compared with solution in each desiccator.

Insects

Adults of *L. brunneus* Steph. were collected by hand soon after emergence from English oak boards, stored under natural conditions in the insectaries at the Laboratory. The beetles were sexed and immediately used for infestation of experimental samples.

Exp. I. The effect of moisture content upon oviposition

One of the $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$ in. conditioned samples was transferred into a small glass tube in each desiccator. Two pairs of *L. brunneus* beetles were added and the tube closed with muslin. The beetles were removed after $4\frac{1}{2}$ days and the number of eggs laid counted with the aid of a binocular microscope, whilst the sample was being shaved away with a razor. The results are given in Table 2, in which the moisture contents are those of the slats during the few days occupied by the experiment.

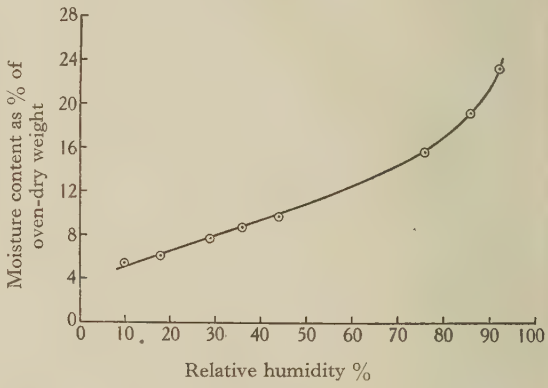

Fig. 1. Relation between expected relative humidity over KOH solutions and equilibrium moisture content of oak sapwood obtained experimentally at 23° C.

Eggs were laid in all samples, but no significance could be attached to the differences in numbers owing to lack of knowledge of the state of the beetles, when collected, with regard to previous oviposition. Beetles may emerge from wood, lay eggs and then crawl back into an old exit-hole, so that a beetle caught leaving an exit-hole is not necessarily newly emerged. It could be concluded with safety, therefore, only that none of the relative humidities experienced by the beetles or the moisture contents of the samples had been sufficiently extreme to prevent egg-laying. This was of interest in relation to Parkin's (1936) observation that beetles would not oviposit in samples lacking starch, indicating, therefore, that beetles do not recognize a low moisture content, at which, as shown later, eggs cannot hatch, as an adverse condition of the wood in the same way as absence of starch.

Table 2. *Results of Exp. I*

Sample no.	Relative humidity %	Moisture content obtained %	No. of eggs
1	10	5·7	44
2	18	6·2	84
3	29	7·8	119
4	36	8·5	24
5	44	9·3	31
6	76	15·8	41
7	86	16·8	45
8	92	23·5	44

Exp. II. The effect of moisture content upon hatching of eggs and early larval development

On the completion of Exp. I, the remaining ½ in. cube of wood in each desiccator was placed in the glass tube and two pairs of beetles added as before. The beetles were removed after 8 days. Seven weeks later the number of tasting marks (Parkin, 1936) and the amount of dust expelled by the young larvae were recorded. The dust was blown off the samples and after a further 4 weeks a second examination was made. The results of these superficial examinations are given in Table 3, in which the moisture content obtained represents the mean of the observations during the 12 weeks' duration of the experiment.

TABLE 3. *Exp. II. Results of superficial examinations*

Sample no.	Relative humidity %	Moisture content obtained %	Tasting marks	Dust expelled After 8 weeks	Dust expelled After 12 weeks
1	10	5·5	35	0	0
2	18	6·1	50	0	0
3	29	7·7	69	+	++
4	36	8·6	49	++	+++
5	44	9·5	91	+++	+++
6	76	15·7	75	++++	++++
7	86	18·0	35	++++	+++
8	92	23·3	78	++	(+)

The large numbers of tasting marks, which appear to be unrelated to the moisture conditions, indicated that eggs had probably been laid in all samples, as was expected from the results of Exp. I. The amounts of dust expelled demonstrated that eggs had hatched in all samples, except the two kept at the lowest humidities, and that 15·7% was near the optimum moisture content for the young larvae.

TABLE 4. *Exp. II. Results of detailed examination*

Sample no.	Moisture content obtained %	No. of larvae	Uninjured larvae No.	Uninjured larvae Weight mg.	Uninjured larvae Mean weight mg.
1	5·5	0*	—	—	—
2	6·1	0†	—	—	—
3	7·7	27	15	4·2	0·280
4	8·6	31	22	8·5	0·386
5	9·5	22	14	7·1	0·507
6	15·7	25	14	9·2	0·657
7	18·0	28	13	5·7	0·438
8	23·3	37	21	4·4	0·209

* 116 unhatched eggs found. † 70 unhatched eggs.

The samples were cut up immediately after the second examination. In spite of every precaution some larvae were cut by the razor, but those uninjured were collected and weighed and the mean weight per larva in the different samples was calculated. The results are set out in Table 4, and the relation between moisture content of the sample and the mean weight per larva is shown graphically in Fig. 2.

The influence of humidity upon the egg period of *Lyctus* is not known, but, on the assumption that the period is not greatly prolonged at either low or high humidities, there is a clearly defined optimum moisture content of the wood for early larval growth in the neighbourhood of 14%. The minimum moisture content for larval growth lies between 7·7 and 6·1%, and by extrapolation would seem to be about 7·0%. Larvae had reached full embryonic development in the eggs in samples 1 and 2, but had died without puncturing the chorion, which had presumably become too hard through desiccation.

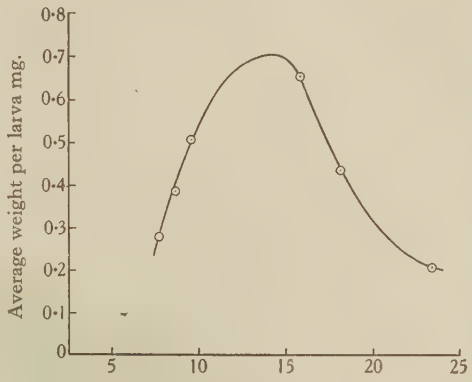

Fig. 2. Relation between moisture content of oak sapwood and mean weight of *L. brunneus* larvae after about 11 weeks at 23° C.

These conclusions may be compared with those of Schuch (1937 *a*, *b*), who worked with larvae of the Cerambycid beetle, *Hylotrupes bajulus*. He found that, at a room temperature averaging 18·8° C., newly emerged larvae died in wood of 7·5% moisture content, but older larvae could survive and increase in weight slowly. As the moisture content rose, the larvae were able to increase in weight more and more rapidly. He found no suggestion of an optimum moisture content below the top of the range investigated, namely, 7·5–24·0%.

Exp. III. The effect of moisture content upon the length of life cycle

At the same time as Exp. II was started, the large blocks of sapwood in the desiccators were exposed to infestation by *L. brunneus*. Three pairs of beetles were placed on each sample, which was then wrapped and tied in muslin. Six weeks later the dead beetles were removed. It was apparent that some of the beetles had become entangled in the muslin and one or two had bitten through it and escaped. These

were probably the chief causes of the small numbers of beetles emerging from some samples, for the second experiment afforded proof that eggs could hatch and larvae develop in wood of moisture content between 7·7 and 23·3 %.

The samples were examined at intervals, until the first exit-hole was observed, and then daily whilst emergence continued. The last beetle recorded emerged 522 days after the start of infestation. Emergence was allowed to proceed naturally and beetles were not forced out of their pupal chambers as soon as the exit-holes were completed. A record was kept of the number of beetles emerging each day, their sex, weight on emergence and weight after drying at 105° C. for 24 hr.: from the two weights, the moisture content of each beetle was calculated as moisture content. The value found for 9·7 % moisture content is considered aberrant.

(*c*) The average weight of the emerging beetles of both sexes was fairly constant up to 9·7 % moisture content, but showed a tendency to rise at higher moisture contents. That of the males was always less than that of the females, but the ranges of weight overlapped considerably.

(*d*) The average moisture content of the male beetles was about 51·5 % up to 15·6 % moisture content of the wood and rose to 59·5 % in the sample of 23·2 % moisture content. The average moisture content of the females tended to rise slowly as the moisture content of the samples rose. The moisture content of the males was consistently higher than that of the females.

TABLE 5. *Data relating to beetles bred at* 23° C. *in oak samples of various moisture contents*

Sample no.		3	4	5	6	7	8
Average moisture content of sample (as % of oven-dry weight)		7·7	8·7	9·7	15·6	19·2	23·2
No. of beetles emerged	♂	17	33	27	7	0	1
	♀	18	24	20	5	3	6
Minimum length of life cycle (days)	♂	286	267	276	167	—	356
	♀	287	274	305	174	196	363
Average weight per beetle (mg.)	♂	2·49	1·86	2·39	2·98	—	4·20
	♀	3·16	2·56	3·58	4·00	3·90	4·95
Average moisture content of beetles (as % of wet weight)	♂	54·2	50·0	51·5	51·0	—	59·5
	♀	43·7	45·3	45·0	47·5	46·2	47·9

a percentage of the wet weight. A summary of the results is given in Table 5, in which the moisture contents of the wood samples are the mean values calculated from the slat weights during the periods from the start of infestation to the date of first emergence from each large block.

No beetles emerged from samples 1 and 2 of average moisture contents 5·5 and 6·1 % respectively, nor were larval tunnels visible when the blocks were sawn across. At the end of the experiment the samples were re-examined for starch; this was found to be present in abundance in all blocks.

The following observations may be made on the figures in Table 5:

(*a*) The numbers of beetles emerging could not be related to the moisture contents of the samples, because of the entanglement of some beetles in the muslin and the escape of others. The small excess of males over females accorded with earlier experience of the sex ratio of this species (Parkin, 1934).

(*b*) The minimum length of life cycle of the males was slightly shorter than that of the females at all moisture contents, and the figures for both sexes indicated a minimum period of development in the region of 15·6 % moisture content. Fig. 3 shows the relation between the moisture content of the wood and the corresponding maximum rate of development for the female beetle, which is plotted as (reciprocal of the minimum length in days of life cycle) × 1000. The curve has its peak at 16 %

Exp. IV. The minimum life cycle of Lyctus brunneus

The period of development from egg to beetle under approximately optimum conditions was ascertained. This experiment was started a year after nos. I–III with a block $4\frac{1}{2} \times 3 \times 2$ in. cut from the same

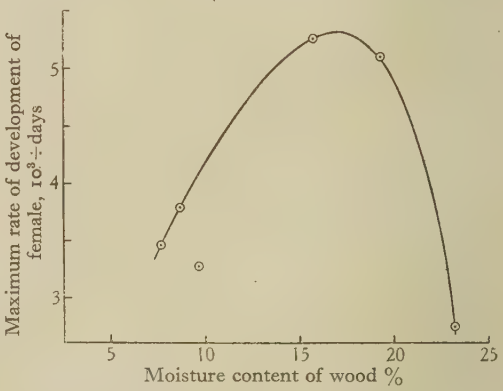

Fig. 3. Relation between moisture content of oak sapwood and maximum rate of development of *L. brunneus* females at 23° C.

air-dry wood: starch was still present in abundance. The block was conditioned at 25° C. and 60 % relative humidity for 6 weeks, after which a slat $\frac{1}{10} \times 3 \times 2$ in. was sawn from one end. The slat was split into two and the moisture content of one-half

was determined, thus enabling the dry weight of the other half to be calculated from its wet weight. The second half was placed with the main block in a desiccator containing KOH solution of a concentration in equilibrium with 70% relative humidity, which according to Fig. 1 is equivalent to 14·2% moisture content. This moisture content was chosen with reference to the results of Exp. II. Exp. III was not completed in time to show that 16% moisture content would have been a better figure.

The desiccator and its contents were maintained at 27·5° C. This temperature was thought to be near the optimum, for 30° C. was known (Parkin, 1934) to be too high for hatching of the eggs, although, in the light of the results of the first experiment of the present series, the failure to hatch may have been due to too low a humidity. After the block had conditioned in the desiccator for 4 weeks, three pairs of *L. brunneus* were put on it and the whole enclosed in muslin as in Exp. III. The beetles were removed after 7 days. The block was examined periodically and the slat weighed at monthly intervals, until the completion of emergence. The mean moisture content of the slat during the period of infestation of the block was 14·2%.

The first small pile of expelled bore-dust was noted 35 days after the start of infestation. The first exit-hole appeared after 130 days, but the beetle, a male, was not found on the surface of the sample until the 142nd day: the first female emerged 4 days later, the exit-hole having been completed on the 140th day. Beetles were found intermittently and, when emergence was complete, the block had yielded a total of 67 (32 males, 35 females) over a period of 121 days: 57 adults, however, were recorded within 56 days of the start of emergence, so that the main period of emergence was about eight times as long as the period of infestation. There was, therefore, considerable variation in the time of development from egg to beetle, even under controlled conditions. The median time of development was 185 days.

Beetles were sexed and weighed, but were not dried for moisture-content determination. The mean weights of the two sexes again showed that the females (3·22 mg.) were significantly heavier than the males (2·33 mg.).

It is evident that the length of life cycle of *L. brunneus* can be reduced from a minimum of about 10 months under natural conditions to 4½ months at 27·5° C. and in timber of 14·2% moisture content. This period of 4½ months is probably not the minimum for development from egg to adult, but is presumably reasonably close to it. A slightly shorter time would almost certainly have been recorded, had the moisture content of the wood been about 16%.

Exp. V. Infestation of newly felled timber

Several pieces of branchwood about 7 in. long and 3 in. diam. were cut in the middle of July from a standing oak. They were brought to the laboratory, barked, sawn lengthwise down the middle and placed in a wood and wire-gauze, insect-proof cage: 17 days later a small number of *L. brunneus* beetles was added. The cage was kept under laboratory conditions during the winter. Examination of the samples one year after the start of infestation disclosed the presence of numerous exit-holes and living and dead *L. brunneus* beetles.

Oviposition by *Lyctus* may, therefore, take place in wood felled only a short while and well before the sample has had time to dry to a mean moisture content below the fibre saturation point. This confirms Fisher's (1928) findings referred to earlier.

Discussion

From the results of the five experiments above, from the scanty literature on this subject and from experience of *Lyctus* infestation under a wide variety of conditions, the susceptibility to *Lyctus* attack of timber at different moisture contents may now be described in general terms.

Newly felled logs with the bark intact are not liable to *Lyctus* attack, until they have been stored for a long time, probably a year or more, since the presence of bark greatly retards the rate of water loss. Wherever small areas of bark have been removed, especially by axe cuts penetrating into the underlying wood, the surface layer of the sapwood may dry sufficiently in the course of a month or two of summer weather to permit oviposition by *Lyctus* beetles. The infestation may then proceed slowly, unless drying has been so slow that depletion of the starch reserves has occurred, in which event the larvae will not survive.

When a log is sawn into boards, a superficial skin of sapwood is soon formed with a moisture content below fibre saturation point, and this peripheral layer becomes liable to attack. During the period of insect development, the skin will be steadily thickening and more wood will become available for larval boring, although the moisture content of a thick board may remain above fibre saturation point for a year or more. Fibre saturation point probably represents the upper limit of moisture content for development of *Lyctus* infestation, for above this point free water will be present in the vessels. As the moisture content decreases, the wood becomes more and more suitable for larval development, until an optimum is reached about 16%. Below this level the wood becomes progressively less suitable again until, beyond some value in the neighbourhood of 7%, development is no longer possible.

During the summer in this country, the moisture content of timber out of doors is very close to the optimum for the development of *Lyctus*. Some degree of control can, therefore, be achieved by keeping timber wetter or drier than about 16% moisture content. Logs with the bark intact may be kept for

a year or more without the moisture content falling sufficiently to permit *Lyctus* infestation, except, very rarely, in a short length at each end. Moreover, during this time progressive depletion of starch from the sapwood will be steadily reducing the liability of the wood to attack. With reference to attack by *Lyctus*, therefore, there is much to be said for keeping timber in the round until required for seasoning, the main drawback of this measure being the probability of infection of the sapwood by wood-destroying fungi.

A moisture content of 7 % can be attained in temperate climates only by kiln-drying the wood, and specially heated or air-conditioned rooms would be necessary for subsequent storage. Many firms already possess kilns capable of drying timber to 7 % moisture content, but, so far as is known, no large-scale storage space exists, at any rate in this country, in which such a low moisture content can be maintained. Veneers are a form of wood which might be suitable for preservation against *Lyctus* damage by storage at a low moisture content, since their thinness would enable the moisture content to be reduced quickly and easily, and their value should make it economic to prepare a small room or chamber in which they could be stored at the required combination of temperature and humidity.

The influence of central heating on the course of *Lyctus* attack in woodwork and furniture in buildings cannot be estimated at present, for too little is known of the combined effect of temperature and moisture content of wood on the development of the insect. It is evident, however, from the data in this paper, that conditions are never likely to become so extreme as to prevent the development of *Lyctus*, except locally, perhaps, in the immediate vicinity of radiators.

On the whole, the general conclusion must be drawn that, in spite of the marked influence of moisture content of wood upon the rate of development of *Lyctus*, control of these insects through control of moisture content cannot be achieved with sufficient facility to make the method of practical importance in any but the most special circumstances.

The work described above has been carried out as part of the programme of the Forest Products Research Board, and is published by permission of the Department of Scientific and Industrial Research. The author was unable to finish the investigation, part of which was completed by Mr E. C. Harris, Technical Assistant, under the supervision of Dr R. C. Fisher, Officer-in-Charge, Entomology Section.

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Experiments on the poisoning of honeybees by insecticidal and fungicidal sprays used in orchards

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(With 2 Text-figures)

Losses of bees by poisoning have been greatly increased in recent years by the growing practice of applying insecticidal and fungicidal sprays to fruit trees. Preliminary laboratory tests showed that, of the common constituents of spray mixtures, only lead arsenate and flowers of sulphur were likely to cause serious honeybee poisoning, though Derris emulsion may cause slight poisoning. Syrup containing lime sulphur, nicotine sulphate, or copper sulphate was strongly repellent to the bees.

It seemed possible that spray mixtures might be made repellent to the honeybee by the addition of suitable substances. Further trials showed that lead arsenate solution, at least in the concentrations normally used, was no more attractive to the bee than distilled water. Concentrations of 1/500 lime sulphur or 1/2000 nicotine sulphate were sufficient to reduce the uptake of *M*/1 sucrose to less than 10 % of that of unadulterated sucrose solution, and very much lower concentrations appreciably affected the uptake. The presence of lead arsenate in these solutions seemed to make them even more repellent. The repellent effect was reduced, though not entirely destroyed, when the solutions were evaporated to dryness and taken up in distilled water again.

Creosote (0·13 %), which has been suggested as a possible repellent, gave erratic results under laboratory conditions, and, in view of the danger of phytocidal action it is probably unwise to attempt to use its repellent property.

Open flowers of apple trees were sprayed with various spray mixtures in an orchard experiment, and counts of bees showed both lime sulphur (1 %) and nicotine sulphate (0·05 % nicotine) to have retained their repellent value for at least 7 days, in spite of heavy rain. If bees can thus be deterred from visiting the open blossom, it should be possible to deter them even more thoroughly from collection of water contaminated by sprays applied at the correct time.

Data collected throughout the country during the last 3 years show calcium or lead arsenate to be the constituent of orchard sprays causing honeybee poisoning. Bees may collect arsenic when visiting fruit trees, or plants growing beneath or near the trees, for pollen or water, contaminated water apparently being the chief cause of poisoning; about 0·00005 mg. of internal arsenic per bee has been found to be a lethal dose. When arsenical sprays alone are applied to trees, particularly if to the open blossom as is sometimes the case with gooseberries and cherries, severe poisoning from the pollen may be expected. On the other hand, much of the danger from contaminated water should be removed by the incorporation of lime sulphur (1 % or more) or nicotine sulphate (0·05 % nicotine) into sprays intended for application either before flowering or after petal fall, provided that alternative sources of water are available for foraging bees. Provision of water within the hive is suggested as a further safeguard.

Since the introduction of extensive spraying of fruit trees complaints of losses of bees from spray poisoning have been increasingly frequent. The subject is important, not only to beekeepers but to all interested in the growing of fruit and seed crops, since data collected during the past 10–15 years show that honeybees are extremely important as agents of pollination.

The present investigation was undertaken to ascertain whether any ingredients of the spray mixtures in general use in this country are attractive or repellent to the honeybee, and with what spray mixtures and under what conditions honeybees are poisoned.

Poison trials

During the summer of 1940 feeding experiments were carried out to determine whether certain of the commoner spray mixtures, or their individual ingredients, are taken by honeybees as food and whether, if taken, they are poisonous to the bees. Observations were made in those cases where poisoning did occur to obtain accurate descriptions of the signs and symptoms of honeybee poisoning by these substances.

Method. One hundred young worker honeybees were isolated in each of six small cages—approximately 5 in. sq.—each being supplied with two small bottle-feeders which the bees could reach through small feed-holes cut in the wooden tops of the cages. In five cages one of these bottle-feeders was filled with *M*/1 sucrose solution and the other with the substance under examination made up in *M*/1 sucrose: 1 % casein had usually to be added to the experimental syrup to obtain an emulsion of the substance under test. It was found that the addition of 1 % casein to *M*/1 sucrose acted neither as an attractant nor as a repellent to the bees. The sixth

cage served as a control, both its bottle-feeders being filled with *M*/1 sucrose.

The cages were kept in the dark at room temperature in the summer. Apart from numerous observations, each cage was examined once every 24 hr. and a count made of the relative numbers of dead and live bees.

In this way the following seven substances were tested:

(1) 1 % casein;
(2) 0·3 % lead arsenate + 1 % casein;
(3) 1 % lime sulphur + 1 % casein;
(4) 0·05 % nicotine sulphate + 1 % casein;
(5) 1 % copper sulphate;
(6) Derris—proprietary, preparation, ¼ lb./3 gall. water;
(7) 2 % flowers of sulphur + 1 % casein.

Results and conclusions. Apart from the obvious cases of poisoning with lead arsenate and flowers of sulphur, there is a slight suggestion of a higher death-rate with the Derris emulsion than in the controls (see Table 1). The bees were observed to take *M*/1 sucrose containing 1 % casein as readily as plain syrup, so that it is unlikely that the addition of this substance to the other materials affected the results.

Table 1. *Results of poison trials conducted by means of feeding experiments*

Substance	No. of cages: Taken freely	Taken slightly	Not taken	% dead after 24 hr.: Substance	Control	% dead after 72 hr.: Substance	Control	% dead after 144 hr.: Substance	Control
1 % casein	5	0	0	0·6	1	8·2	6	17·8	16
0·3 % lead arsenate	5	0	0	94·0	2	99·8	8	100·0	18
1 % lime sulphur	0	0	5	3·2	4	9·4	5	16·2	16
0·05 % nicotine sulphate	0	1	4	2·0	0	6·8	6	13·6	16
1 % copper sulphate	0	0	5	3·4	2	7·6	9	15·0	15
Derris emulsion	0	5	0	2·6	2	10·2	3	20·4	14
2 % flowers of sulphur	5	0	0	85·2	2	97·6	9	99·8	16

Definite signs of acute poisoning by lead arsenate were observed 1 hr. after feeding. Half an hour after this experiment was set up large numbers of 'crawlers' appeared on the bottom of each of the experimental cages. The legs and wings of these 'crawlers' appeared to be partially paralysed and were frequently held in unnatural positions while considerable trembling occurred. The abdomens of affected bees became greatly distended before death, and dysentery was apparent.

With the Derris emulsion it was believed that a trace of poisoning occurred, although no definite symptoms were observed other than slight dysentery. This substance appeared to be taken only sparingly by the honeybees and to be somewhat repellent.

As previously observed by Herman & Brittain (1928–32), syrup containing flowers of sulphur was taken greedily by the bees. Little evidence of poisoning was apparent after 6 hr., although a few bees appeared to have slightly abnormally distended abdomens and these individuals constantly made cleaning movements with their legs. After 12 hr. there were a number of dead bees; this number increased very rapidly during the next few hours, the individuals concerned having distended abdomens and performing cleaning movements before dying. Sulphur fed in this way thus appears to be almost as poisonous to bees as arsenic, although death does not take place so rapidly.

A high degree of repellence was noted with lime sulphur, nicotine sulphate, and copper sulphate at the strengths used in most of the experiments. Since these substances are commonly used in spray mixtures, it appeared worth while to study in detail their possible repellent effect on the honeybee, both in the laboratory and in the field.

Laboratory experiments on possible repellents

Method. Emulsions of the various substances to be tested were freshly prepared in *M*/1 sucrose using 1 % casein as an emulsifying agent. Measured quantities (203 mg.) of the emulsions were placed in individual cells in a piece of dry brood comb. (Fig. 1 shows the apparatus for this purpose.) Several different substances, or different concentrations of the same substance, were used simultaneously in an experiment, replicate cells of the different treatments being randomized so as to permit the elimination of position effects. In each experiment sufficient cells were filled with plain *M*/1 sucrose to ensure that there was always a surplus of this substance present, since it was found that starving bees will brave even strong repellents such as citronella oil in order to obtain nourishment. The comb in which the different treatments had been laid out was placed in a large circular experimental chamber approximately 12 in. diam. and 9 in. deep, through which a stream of warm air (25–30° C.) was continuously drawn by means of a filter pump. One hundred or more adult bees, taken from a well-stocked colony immediately before an experiment, in order to be certain that they were neither starving nor with full honey-stomachs, were then placed in this apparatus and allowed to feed. The number of bees used in each experiment was determined by the number of experimental cells used. After 1 hr. or more the bees were all collected

and the amount of the substance remaining in each cell measured by means of the micro-pipette shown in Fig. 2. Care was taken to determine the approximate loss of volume by evaporation during the course of an experiment in order that corrections might be applied.

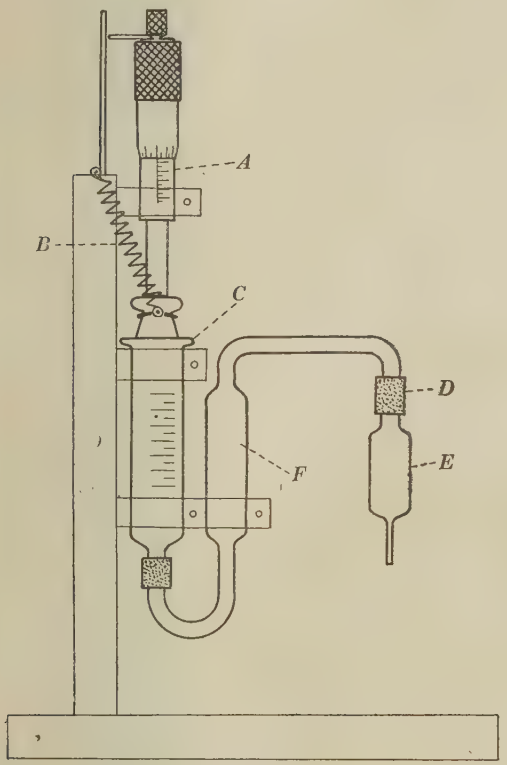

Fig. 1. Micrometer-controlled delivery syringe for placing a predetermined quantity of fluid into a cell in a comb. *A*, micrometer; *B*, return spring; *C*, syringe; *D*, rubber connection; *E*, delivery reservoir; *F*, displacement reservoir filled with water.

Results. Statements have frequently appeared in the Bee Press to the effect that honeybees are attracted to sprayed trees because the arsenic employed in the spray mixtures appears to them to have a sweet taste. Tests were therefore made to determine whether arsenic, at the strength usually employed in spray mixtures, exerts any attraction for the honeybee. Table 2 shows the result obtained with 0·3 % lead arsenate paste (15 % As_2O_5 w/w).

The uptake of sucrose was not appreciably affected by the presence of lead arsenate, but in the absence of sucrose little lead arsenate was taken. This shows the lead arsenate itself to have had little attraction for the bees as long as sucrose was plentiful. Half the bees in the experiment were dead after 12 hr.

In a second experiment, the results of which are given in Table 3, lead arsenate was tested against a series of low concentrations of sucrose. Again there was adequate *M*/1 sucrose available and consequently little inducement for the bees to take the less concentrated solutions. The lead arsenate proved no more attractive than 0·003 % sucrose, which itself showed only the same uptake as distilled water.

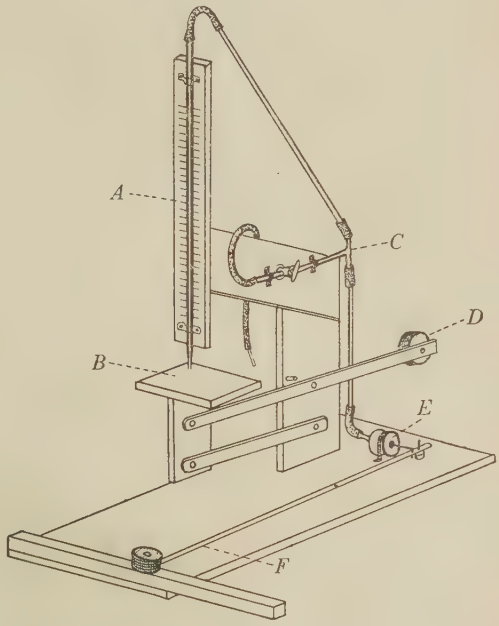

Fig. 2. Micro-measuring pipette for determining the quantity of fluid remaining in an individual cell in a piece of brood comb at the end of an experiment. *A*, micro-pipette calibrated in mg. of molar sucrose; *B*, platform on which comb is placed; *C*, T-piece to allow of oral suction for cleaning purposes via tap and mouthpiece; *D*, counterpoise weight to platform; *E*, rubber diaphragm, displacement of which controls the movement of fluid into or out of the pipette; *F*, lever for actuating rubber diaphragm.

Higher sucrose concentrations were taken to a slightly greater extent, but not in amounts comparable with the *M*/1 solution.

Table 2. *Results of an experiment to determine whether lead arsenate is attractive to the honeybee*

Solution	Mean uptake (mg./cell, corrected for evaporation)
M/1 sucrose	76
M/1 sucrose + 0·3 % lead arsenate paste	74
0·3 % lead arsenate paste	8

100 bees for 1½ hr., with 21 cells of each solution.

The experiments in 1940 suggested that lime sulphur and nicotine sulphate were repellent to the honeybee. Experiments employing the same ap-

paratus as for lead arsenate were used to test the effect of different concentrations of these substances on the attractiveness of *M*/1 sucrose solution. It was found that a concentration of 1/500 lime sulphur or 1/2000 nicotine sulphate was sufficient to reduce the uptake of sucrose to less than 10% of that of unadulterated solution. The uptake steadily increased with decreasing concentration, but as little as $1/1{\cdot}5 \times 10^6$ lime sulphur or $1/30 \times 10^6$ nicotine sulphate was sufficient to cause a substantial reduction in the uptake. Over the range of concentrations tested an average reduction of uptake per cell for each five-fold increase in concentrations by $12{\cdot}6 \pm 1{\cdot}3$ mg. in the case of lime sulphur and $7{\cdot}6 \pm 1{\cdot}4$ mg. in the case of nicotine sulphate adequately represented the results shown more fully in Tables 4 and 5.

TABLE 3. *Comparison of the attractiveness of lead arsenate and various concentrations of sucrose*

Solution	Mean uptake (mg./cell, corrected for evaporation)
M/1 sucrose	192
0·3 % sucrose	15
0·15 % sucrose	17
0·03 % sucrose	18
0·015 % sucrose	17
0·003 % sucrose	12
0·3 % lead arsenate paste	12
Distilled water	12

100 bees for 2 hr. with 7 cells of each solution.

TABLE 4. *The effect of different concentrations of lime sulphur on the attractiveness of M/1 sucrose*

Solution	Mean uptake (mg./cell, corrected for evaporation)
M/1 sucrose	90
M/1 sucrose + 1/1,562,500 lime sulphur	69
M/1 sucrose + 1/312,500 lime sulphur	69
M/1 sucrose + 1/62,500 lime sulphur	63
M/1 sucrose + 1/12,500 lime sulphur	35
M/1 sucrose + 1/2,500 lime sulphur	25
M/1 sucrose + 1/500 lime sulphur	8
M/1 sucrose + 1/100 lime sulphur	5
Standard error	±7

100 bees for 2 hrs. with 8 cells of each solution.

A direct test of the apparent repellents in the presence of lead arsenate was next made. To an *M*/1 sucrose solution was added 0·3% lead arsenate paste: different portions of this were then made up containing all combinations of presence or absence of 1% lime sulphur and presence or absence of 0·05% nicotine, either as nicotine or as nicotine sulphate. In order to simulate orchard conditions, the six solutions were evaporated to dryness at room temperature with a fan and, on the following day, were taken up again in the correct volume of distilled water and used in the experiment. Adequate supplies of unadulterated *M*/1 sucrose were available for the bees throughout the experiment.

TABLE 5. *The effect of different concentrations of nicotine sulphate on the attractiveness of M/1 sucrose*

Solution	Mean uptake (mg./cell, corrected for evaporation)
M/1 sucrose	62
M/1 sucrose + 1/31,250,000 nicotine sulphate	55
M/1 sucrose + 1/6,250,000 nicotine sulphate	33
M/1 sucrose + 1/1,250,000 nicotine sulphate	31
M/1 sucrose + 1/250,000 nicotine sulphate	26
M/1 sucrose + 1/50,000 nicotine sulphate	17
M/1 sucrose + 1/10,000 nicotine sulphate	13
M/1 sucrose + 1/2,000 nicotine sulphate	4
Standard error	±7

100 bees for 2 hr. with 8 cells of each solution.

The results (Table 6) show practically no difference between nicotine and nicotine sulphate, both having a strongly repellent action. Lime sulphur alone also reduced the uptake by the bees, though not to the same extent as the nicotine, and the two substances together did not give any greater repellence than nicotine alone. In this experiment lead arsenate itself slightly reduced the sucrose uptake.

TABLE 6. *The effect of lime sulphur and nicotine, alone and in combination, on the uptake of M/1 sucrose containing lead arsenate*

Mean uptake (mg./cell, corrected for evaporation) ±1·9

	No nicotine	Nicotine	Nicotine sulphate	Mean (±1·1)
No lime sulphur	33·5	1·8	3·7	13·0
Lime sulphur	7·7	2·8	3·0	4·5
Mean (±1·4)	20·6	2·3	3·4	8·8

Unadulterated *M*/1 sucrose: Mean uptake 39·7 mg./cell. 100 bees for 1½ hr. with 6 cells of each solution.

A similar experiment was designed to show the effect of lead arsenate on the repellent action of lime sulphur and nicotine. *M*/1 sucrose solutions containing lime sulphur (1%), nicotine, or nicotine sulphate (0·05% nicotine) were made up both with and without 0·3% lead arsenate paste. The same procedure with regard to evaporation and taking up again was followed.

Table 7 shows nicotine itself to have had no repellent action in the absence of lead arsenate, though nicotine sulphate was still effective. Lead arsenate seemed to make all three substances more repellent, though only with nicotine was the effect perfectly clear. Even in the presence of lead arsenate, nico-

tine was less repellent than nicotine sulphate, but, in view of the high variability and the contradictory evidence of the preceding experiment, it would be unwise to attach significance to this result.

TABLE 7. *The effect of lead arsenate on the repellent action of lime sulphur and nicotine*

Mean uptake (mg./cell, corrected for evaporation) ±4·0

	Lime sulphur	Nicotine	Nicotine sulphate	Mean (±2·3)
No lead arsenate	5·8	39·0	8·0	17·8
Lead arsenate	1·3	9·2	2·7	4·1
Mean (±2·8)	3·0	24·1	5·4	10·8

Unadulterated *M*/1 sucrose: Mean uptake 38·2 mg./cell. 100 bees for 1½ hr. with 6 cells of each solution.

In a final laboratory experiment an attempt was made to compare a number of different combinations of substances with lead arsenate. Six mixtures were prepared consisting of lead arsenate with or without lime sulphur and with nicotine, with nicotine sulphate or without any form of nicotine. This number of treatments was doubled by the introduction of a creosote versus no creosote comparison and all were tested both freshly made up and retaken up in water after evaporation. All contained *M*/1 sucrose. Two additional treatments were creosote with *M*/1 sucrose alone and *M*/1 sucrose alone (both without lead arsenate). The standard quantities used were 0·3 % arsenate paste, 1 % lime sulphur, 0·05 % nicotine and 0·13 % creosote. Unfortunately, the nicotine sulphate was used at a rate of approximately 0·01 % nicotine instead of 0·05 %, and the results are therefore scarcely comparable.

The more important aspects of the results of this experiment are summarized in Table 8. The first section shows the effect of the evaporation and subsequent retaking up of the three nicotine treatments averaged over all combinations of lime sulphur and creosote. In all cases the repellence was substantially reduced after evaporation. The second section shows the average effect of creosote; the reduction in uptake was small in the presence of nicotine but larger with nicotine sulphate or without any form of nicotine. Next is shown the average effect of lime sulphur, the response to which was much reduced by the presence of nicotine or nicotine sulphate. Both nicotine and nicotine sulphate reduced the uptake per cell, though, on account of the mistake in quantities, the latter was appreciably less effective.

Perhaps the most interesting aspect of this experiment is that shown in the final section of Table 8. Bearing in mind the phytocidal action of creosote, it is probable that its repellence of the honeybee is not sufficient to justify its use in spray mixtures. The effects of lime sulphur and nicotine are therefore shown for the mixtures which did not contain creosote and, in order to be nearer to field conditions, only the results after evaporation and retaking up in solution are used. The figures should therefore be directly comparable with those given in Table 6. Except for nicotine sulphate, the two tables agree reasonably well. The uptake was much greater in the present experiment, but in both cases lime sulphur or nicotine successfully reduced the uptake by a large amount, the two together having no appreciably greater effect than either alone.

TABLE 8. *Effects of creosote, lime sulphur, and nicotine on the uptake of M/1 sucrose containing lead arsenate*

Mean uptake (mg./cell, corrected for evaporation)

Sucrose alone	91
Sucrose + creosote alone	29

All remaining treatments contain lead arsenate and *M*/1 sucrose.

(i) Averages for lime sulphur and creosote (±2·0)

	No nicotine	Nicotine	Nicotine sulphate	Mean (±1·1)
Fresh	33	10	14	19
After evaporation	59	31	44	45
Mean (±1·4)	46	21	29	32

(ii) Averages for lime sulphur and fresh versus evaporated (±2·0)

	No nicotine	Nicotine	Nicotine sulphate	Mean (±1·1)
No creosote	58	21	39	39
Creosote	34	20	19	24
Mean (±1·4)	46	21	29	32

(iii) Averages for creosote and fresh versus evaporated (±2·0)

	No nicotine	Nicotine	Nicotine sulphate	Mean (±1·1)
No lime sulphur	66	27	39	44
Lime sulphur	26	15	19	20
Mean (±1·4)	46	21	29	32

(iv) Averages *without* creosote and *after* evaporation only (±4·7)

	No nicotine	Nicotine	Nicotine sulphate	Mean (±2·4)
No lime sulphur	113	24	95	77
Lime sulphur	18	22	49	30
Mean (±3·3)	66	23	72	54

400 bees for 2 hr. with 6 cells of each of the 24 solutions containing lead arsenate, 24 cells of creosote + sucrose alone and of sucrose alone.

ORCHARD EXPERIMENTS

Earlier experiments having indicated that the attractiveness of a sucrose solution and the repellent action of lime sulphur or nicotine sulphate were not greatly affected by the presence of lead arsenate, it was decided to test these repellent actions under field conditions in the orchard, but to avoid undue risk to the bees by omitting lead arsenate from the spray mixture.

Six spray mixtures were tested, each containing

1% casein as an emulsifier. No sucrose was used. The mixtures were casein alone, casein with 0·05% nicotine, casein with nicotine sulphate to the amount of 0·05% nicotine, and each of these three with 1% lime sulphur. Unsprayed branches were used as controls.

On each of seven apple trees in the experimental orchard three branches, having sixty-five bunches of flowers each, were selected for the experiment and marked. The three branches on each of the trees were then assigned to three of the seven treatments (six sprays and control) in such a way that each treatment was used three times in all, and every pair occurred together on the same tree once only. By balancing the treatments in this way any differences between trees in their attractiveness to the honeybee could be eliminated from the subsequent analysis.

The sprays were applied to the appropriate portions of the trees between 10.00 and 11.00 hr. G.M.T., on 14 May 1942, the day being deliberately chosen when the flowers were open.* Counts of honeybees present on each of the twenty-one experimental branches were then made at frequent intervals on that and every subsequent day until 27 May. Weather conditions during this period were as follows:

14 May Overcast at first, mostly sunny, cloudy at times p.m. Cloudy night.
15 ,, Dew and mist early, cloudy with frequent bright intervals, overcast at night.
16 ,, Rain early, mostly sunny with frequent cloudy intervals, clear night.
17 ,, Sunny a.m. becoming cloudy, showers. Shower early p.m. Rain at night.
18 ,, Little rain early, mostly cloudy during day, occasional rain in evening.
19 ,, Little cloud at first, sunny rest of day.
20 May Sunny a.m. becoming overcast p.m., rain in evening.
21 ,, Light drizzle at first, cloudy day, little sun p.m.
22 ,, Mostly cloudy, occasional heavy rain p.m. with thunder and lightning.
23 ,, Sunny early a.m. becoming overcast. Continuous rain p.m.
24 ,, Sunny a.m., cloudy with bright periods p.m., rain in evening.
25 ,, Dull day, heavy rain and high wind p.m.—clearing later.
26 ,, Bright periods early, mostly cloudy during day, heavy rain p.m.—clearing later.
27 ,, Sunny a.m., cloudy p.m. Rain at night, high wind.

* It is not recommended that open blossom should ever be sprayed in practice. Nor is it desirable that honeybees should be repelled from open fruit blossom since they are in many districts very largely responsible for its pollination. In this instance, for experimental purposes, open fruit blossom was sprayed with supposed repellents in order that their value under orchard conditions might be measured.

The total number of bees on the 195 bunches of flowers receiving each treatment is shown in Table 9; these totals have been adjusted to eliminate differences between trees, and averaged over the several occasions on which counts were made. It is clear that all the five repellent mixtures succeeded in keeping away the bees on the first day. On the following day the effectiveness of nicotine alone had almost entirely disappeared, and from that day onward as many bees visited flowers sprayed with nicotine as visited those sprayed with casein alone. The effect of nicotine sulphate, however, was unimpaired. Not until 18 May did the other four spray treatments begin to lose their effect, though rain had fallen as early as 16 May. From 21 May onward there was no apparent effect of any of the sprays. Lime sulphur was the most consistently successful repellent, though the difference between this and nicotine sulphate was very small. There was no evidence that either nicotine or nicotine sulphate added to lime sulphur had any effect on its repellent powers.

TABLE 9. *Orchard-spraying trial to test the repellent action of lime sulphur, nicotine, and nicotine sulphate*

	Av. no. of bees on 195 bunches of flowers						
			Sprayed with casein +				
Date	Unsprayed	Sprayed with casein	Lime sulphur	Nicotine	Nicotine sulphate	Lime sulphur + nicotine	Lime sulphur + nicotine sulphate
14 May	21	21	0	0	1	0	0
15	14	15	0	12	0	0	0
16	7	7	0	6	0	0	1
17	9	8	0	10	0	1	0
18	23	24	5	25	9	7	6
19–20	16	12	3	16	6	3	6
21–23	10	13	11	12	10	10	11
24–27	10	11	10	8	10	10	10

GENERAL DISCUSSION

A. *Poisoning agent and lethal dose*

Study of a number of cases of suspected poisoning during the past 3 years have indicated that only those sprays containing arsenic in the form of lead or calcium arsenate cause serious poisoning of honey-

bees in the field. No clear cases of poisoning with nicotine, nicotine sulphate, lime sulphur or copper sulphate have been obtained, either in the laboratory or in the field. This is in general agreement with the conclusions of Herman & Brittain (1928–32). Bourne (1927), on the other hand, found that lime sulphur, lead arsenate and nicotine sulphate when mixed with honey were each poisonous to the honeybee in the laboratory; it should be noted, however, that he used the powerful attractant honey in his experiments and that an alternative source of uncontaminated food was not provided.

Various workers have conducted experiments, both in the laboratory and in the field, to determine the lethal dose of soluble arsenic necessary to kill an adult honeybee. Price (1920) found that a fatal dose for a bee is less than 0·0005 mg. As_2O_5. McIndoo & Demuth (1926) established that a fatal dose of arsenic per bee is about 0·0004–0·0005 mg., and Hilgendorff & Borchert (1926) found up to 0·004 mg. of internal arsenic per bee in bees that had died from spray poisoning. Herman & Brittain (1928–32) reached the conclusion, from extensive laboratory and field experiments, that poisoning may be suspected when the internal arsenic found in adult bees is greater than 0·00004 mg. per bee, and that poisoning has definitely occurred when more than 0·00008 mg. of arsenic per bee is present. Analysis, by means of the Gutzeit method, of bees killed during the poison trials described in the present paper showed that the internal arsenic in these bees ranged from 0·00004 to 0·003 mg. per bee. It therefore seems safe to conclude that when the internal arsenic per bee is found to be higher than 0·00005 mg. poisoning has occurred. The solubility of lead arsenate in the insect gut has been discussed by Tietz (1924) and Fink (1926) among other workers.

B. *Collection of poison sprays by honeybees*

It appears from field observations that the great majority of bees which collect arsenical sprays die in the field and never regain the hive if this is situated at some distance (½–1 mile) from the source of poisoning. When the colonies of bees are near or in the orchard concerned, dead and dying bees are likely to be found around the hives themselves. This would be explained by the fact that, as shown in the experiments described earlier, a bee that has collected arsenic and become poisoned usually becomes a 'crawler' (i.e. more or less incapable of sustained flight) within a short time. Actual death occurs at any time between 1 and 12 hr. later according to the degree of poisoning. Poisoning usually results in severe weakening of the colony concerned rather than in its complete destruction.

Poisoning may be of two main types: that resulting from the collection of the spray mixture itself, and that from the collection of contaminated pollen.

The experiments described above (Table 3) have shown that, contrary to statements frequently made, in the concentrations usually employed for spray mixtures arsenic is no more attractive to the honeybee than is distilled water. Both in this country and elsewhere (Herman & Brittain, 1928–32) it has been shown that the honeybee collects arsenical mixtures 'accidentally' when seeking drinking water, not when seeking nectar. Since spray mixtures are somewhat hygroscopic and honeybees frequently collect their drinking water from the foliage and trunks of trees, they are likely, unless more favourable sources of water are readily available or the spray mixtures contain some deterrent, to collect their water from sprayed trees situated near the hives, incidentally collecting arsenic. As might be expected, poisoning of this type appears, from the available data, to be more prevalent in dry weather.

Contaminated pollen may cause poisoning as a result of the fruit trees being sprayed while the flowers are open or, perhaps more frequently, as a result of spray applied at the pink-bud stage or as a calyx spray falling or drifting on to other flowering plants in or near the orchard (dandelion, hawthorn, etc.). In orchards containing mixed varieties, which come into flower at different times, the sprays are sometimes applied when some trees have open flowers. Gooseberries and cherries also are sometimes sprayed, often with an arsenical alone, while the flowers are still open. Under these conditions bees frequently collect large quantities of pollen contaminated with arsenic and return to the hive with it, since they generally eat too little while foraging to die in the field. This pollen may be stored in the hive for some time before being eaten by adult bees or fed to the brood. As a consequence, unlike the poisoning resulting from contaminated drinking water, poisoning of adult bees of all ages and of their brood may take place and continue for some time after collection of the pollen has ceased.

Herman & Brittain (1928–32) made a large number of analyses of nectar taken from colonies poisoned by arsenical sprays but found either no arsenic or only minute amounts. This is in agreement with the relatively small number of analyses made during the course of the present investigation, no arsenic ever being found in nectar or honey stored in the hive.

C. *Repellent action of spray mixtures*

McIndoo & Demuth (1926) found that lime sulphur was repellent to the honeybee; Bourne (1927) found nicotine also to be repellent. Herman & Brittain (1928–32) found, in the case of bees enclosed in cages with fruit trees, that generally, but not invariably, less toxicity was exhibited by sprays containing nicotine or copper sulphate as an ingredient. They concluded that repulsion from the regular lime-sulphur-arsenate spray is likely to be temporary and of little significance under field conditions. The addition of nicotine appeared to increase

the repulsion. On the other hand, in feeding tests these workers found that copper sulphate, lime sulphur or nicotine sulphate, incorporated in sugar syrup in the concentrations usually used in spraying, rendered the syrup so strongly repellent that the bees refused to feed on it. The latter observations are in agreement with those recorded in this paper. Unlike the results obtained in Herman & Brittain's tent experiments in the orchard, however, in the present investigations these substances were found to exert a powerful repellent influence for some days even when applied to apple trees that were in flower. This is in agreement with recent observations on the use of lime sulphur as a repellent (Anon. 1941) in America, where very severe losses from spray poisoning have been reported. When 1 % or stronger lime sulphur was employed in spray mixtures honeybee mortality was very greatly reduced. Creosote at a strength of 1 pt./100 gal. of spray mixture was also claimed to give successful results although some scorching of the foliage occurred. Tests with creosote as a repellent gave rather erratic results under laboratory conditions (Table 8).

Conclusions

No cases of poisoning of honeybees by orchard sprays other than those containing arsenic, in the form of calcium or lead arsenate, have been reported. Judging by the results obtained in the experiments described in this paper the use of 1 % or stronger lime sulphur or 0·05 % or stronger nicotine sulphate in conjunction with lead arsenate should repel the great majority of bees from sprayed trees and thus prevent them from being poisoned, provided that other sources of pollen, water and nectar are readily available. In at least two of the severe cases of arsenical poisoning of bees in the field investigated in 1942 an arsenical spray alone was applied to the trees. On the other hand, in all the experiments described above there was always an alternative source of nectar (or syrup) available for the bees, and in the orchard experiments there were also natural supplies of nectar, pollen and water. Had there been no alternative sources of food for the bees, within easy flying distance, experience with laboratory experiments indicates that the foraging bees might quite well ignore the repellent nature of the spray. A starving honeybee will, in fact, take syrup containing well-known repellent substances.

It would appear, therefore, that when bees have alternative sources of water, pollen, and nectar, the addition to spray mixtures of lime-sulphur or nicotine sulphate will reduce the risk of arsenical poisoning. Preliminary trials have shown that water can best be given in the hive itself by means of 'frame-feeders', or alternatively, though not quite so successfully, in an ordinary 'rapid-feeder' placed over the frames. The provision of water inside the hive is likely not only to lessen the risk of bees collecting poisoned drip-water from sprayed foliage, but also to conserve the energy of the colonies concerned. Probably it will prove best to supply a small quantity of sugar in the feeding water in the first instance in order to encourage the bees to collect it, but this can be dispensed with after the first few days.

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Field studies of potato-root eelworm, *Heterodêra rostochiensis* Wollenweber, 1938–40

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Field studies of potato crops under different soil and fertility conditions and exposed to attack by diverse concentrations of potato-root eelworm led to the following conclusions:

In the north-western agricultural advisory province potato-root eelworm is not a major cause of failure or partial failure of potato crops on land where the viable cyst concentration is low (under 10/10 c.c. of soil). On such land increased yields can be obtained by improving conditions for plant growth, widening the rotation and increasing the fertility of the soil.

The production of satisfactory crops on land with a low eelworm population is likely to be accompanied by considerable increases in the numbers of root eelworms in the soil.

Crops free from signs of 'potato sickness' or 'potato-root eelworm disease' can be grown on land heavily infested with root eelworms (viable cyst concentration exceeding 25/10 c.c.), if no other adverse condition, such as unsuitable weather, lack of available food or incidence of fungus disease, affects the growth of the crop, but yields from heavily infested land are not likely to be more than moderate.

The treatments used against eelworms in the trials described here did not give results commensurate with their cost.

The practical difficulties of incorporating lethal substances with soil in the field are considerable, and this may have contributed to the disappointing results obtained.

The study of the influence of host variety on cyst production suggests that varietal differences in the host plants may influence the numbers of cysts formed on the crop, but the evidence is not conclusive.

The persistent low yields of potatoes and the wide distribution of potato-root eelworm present a serious problem in the peat-moss areas of the Mersey basin where potatoes are a staple crop. Local interest was aroused in 1937 when Mr J. Albinson, B.Sc., of the Manchester Corporation Gas Works, suggested that gas-works liquor, a waste product in the manufacture of gas, might be of value in checking eelworm disease, and potato plants in portions of fields to which gas-works liquor was applied were found to be more vigorous and healthy than the surrounding crops. With the aid of grants from the Agricultural Research Council, Manchester Corporation and local branches of the National Farmers' Union, trials were arranged to test the value of gas-works liquor at centres where different conditions of soil, fertility and eelworm intensity occurred, and to compare its effects on the potato crop and the eelworm parasites with those of calcium cyanamide and calcium chloroacetate. Several varieties of potato were used since Robertson (1937, 1939) had suggested that varietal differences might influence the intensity of eelworm invasion. The trials were repeated in order to note the cumulative effects of treatment and the effects of successive crops on plant health and eelworm intensity. Manurial trials were arranged on land where potato sickness was known to be severe.

Description of experiments

At centres 1–4 areas of 800 sq. yd. were divided into sixteen equal plots on a 4×4 factorial plan. In 1938 treatment and variety were assigned to the plots as a complex Latin square, and the sixteen combinations of treatment and variety occurred only once at each centre. In 1939 one variety of potato was used throughout the trial; soil treatments were continued and the same plot arrangement was followed so that each plot had the same treatment for two consecutive seasons. In 1940 the same plot arrangement was maintained and the four varieties selected in 1938 were again grown; the treatments were discontinued but the plots at all four centres received the same manurial dressing.

Varieties selected were Arran Banner, Majestic, Great Scot and Doon Star. All are popular in Lancashire and Cheshire and great differences between them were not expected. Substances used as treatments were gas-works liquor, calcium cyanamide, and commercial calcium chloroacetate. The gas-works liquor consisted of:

Ammonia (free) 0·63 g./100.
Ammonia (fixed) 0·83 g./100.
Phenols as C_6H_5OH 0·70/100.
Sulphides (H_2S) 0·02 g./100.
Thiocyanates as CNS 0·04 g./100.
Thiosulphates as S 0·05 g./100.

It was applied at the rate of 2 gal./sq. yd., thus adding to the soil the equivalent of 40 cwt./acre of sulphate of ammonia. It was expected that this would act as a partial sterilizer and as a stimulant to growth. Calcium cyanamide was used at 3 cwt./acre; it is a nitrogenous fertilizer and has an injurious effect on potato-root eelworm (Edwards, 1937; Hurst & Triffitt, 1935, 1937; Hurst & Franklin, 1937; Miles, 1930). Commercial calcium chloro-

acetate was used at 3 cwt./acre; it has no special value as a fertilizer but has given promising results against eelworms (Smedley, 1938; Edwards, 1939; O'Brien *et al.* 1939).

At centre 5 in 1939 the same four varieties were grown in combination with dressings of calcium cyanamide at 3 cwt./acre and 2 tons/acre. The experiment was arranged as a randomized block of forty-eight plots, each 40 sq. yd., and each variety-treatment combination occurred four times. In 1940 the same varieties were grown in conjunction with manurial dressings of (1) farmyard manure 21 tons/acre supplemented with complete fertilizer 4 cwt./acre, (2) complete artificial fertilizer 8 cwt./acre, and (3) slaughter-house refuse 30–35 tons/acre. The plots were arranged as three randomized blocks in which each variety-treatment plot (40 sq. yd.) occurred once.

At centre 6 a variety trial was arranged as a Latin square on a plot of 800 sq. yd. Arran Pilot, Ninety-fold and Duke of York were selected because they were widely grown in the area, and Epicure was included at the suggestion of Dr Robertson.

Notes on conditions at the centres

Centre 1 was on peat moss, and a large part of the soil consisted of peat fragments. The land was well worked and in a high state of fertility, and the crops included market-garden produce, potatoes and, at intervals, oats and clover. In 1938 the viable cyst concentration was 34/10 c.c. Centre 2 was also on peat moss, but the land was in poor condition and the state of fertility was unsatisfactory. Cropping was similar to that of centre 1. Signs of severe potato sickness had frequently been observed on the field, and the viable cyst concentration was 7/10 c.c. Centre 3 was on peat moss and also in poor condition. Potato sickness was prevalent on the farm, and for some years potato crops had been poor. Recently the farming system had been changed from intensive market gardening to a modified four-course rotation which included potatoes. The viable cyst concentration was 8/10 c.c. Centre 4 was on light, sandy soil, badly drained and in poor condition. Market-garden crops and potatoes were usually grown, and cereals and one-year leys occurred occasionally. The viable cyst concentration was 4/10 c.c. Centre 5 was on peat moss and in a poor, farmed-out condition. Though the prevalence of potato sickness had for many years made potato crops unprofitable they were still regularly grown, and the market-garden crops usually produced on the holding were well below the standard for the district. The viable cyst concentration was 5/10 c.c. Centre 6 was on peaty sand. Severe potato sickness had caused the failure of the potato crop on this field in 1928, but after that time the farming system was modified and the intervals between potato crops were increased. When the field was sampled the total cyst population was 2/10 c.c., which, since cysts are known to persist for many years (Miles, 1942), suggested that when the crop failed in 1928 the eelworm population could not have been high. At the time of planting in 1940 the viable cyst concentration was very low.

Measurement of results

Crop yields and numbers of viable cysts were used as indicators. Morgan's flotation method was used to separate cysts from air-dried, sieved soil, and the spherical cysts of *Heterodera rostochiensis* were isolated from other species present. At centres 1–5 the unit volume of soil adopted was 10 c.c. The use of a volume unit is open to criticism, as small volumes of peaty soil vary in weight owing to differences in particle size affecting compactness, but since a plant is generally considered as growing in a volume of soil the cysts from a volume unit seemed to indicate most satisfactorily the degree of infestation. At centre 6, where the soil had a large proportion of sand and the eelworm population was low, the unit volume used was 50 c.c., but for convenience the viable cyst concentration per 10 c.c. is recorded (Table 6).

At centres 1–5 cysts were counted from six samples of 10 c.c. of soil. The standard error varied inversely with the numbers of cysts present. In series where the population was about 75/10 c.c., the standard error was approximately 4%, and in series with a cyst population of about 30/10 c.c. the standard error was approximately 7%. These errors are of a similar order to those recorded by Smith & Prentice (1929) in their study of the errors involved in the technique of estimating eelworm infestation.

In Tables 1–7 the viable cyst concentration in soil samples taken shortly after the crop was lifted is used as an indicator of the extent of eelworm parasitism in the crop preceding the sampling. That this was the most suitable indicator was suggested by the observation that at centres where the numbers of viable cysts decreased from one year to another there was an increase in the total number of cysts, and this increase, after making allowance for error, could generally be associated with the number of viable cysts in the second of the two years under consideration. Further, Triffitt (1930) concluded that approximately all the eggs contained by overwintered cysts of potato-root eelworm were capable of hatching during the first season following their development; and observations made by the authors over a series of years on the condition of matured cysts at different points in the crop rotation and also during the growth of the host plants, have indicated that the source of practically all cysts classed as viable was the potato crop that preceded sampling.

Table 1. *Treatment-variety trial,* 1938. *Yield (lb./plot)* and viable cyst concentration after cropping (per 10 c.c. soil)*

Centre	Treatment	Arran Banner		Majestic		Great Scot		Doon Star		Average	
		Yield	Cysts	Yield	Cysts	Yield	Cysts	Yield	Cysts	Yield	Cysts
1	Gas-works liquor	175	32	145	34	157	73	151	42	157	45
	Calcium cyanamide	186	48	109	32	108	32	86	34	122	36
	Calcium chloroacetate	203	46	111	24	172	42	120	21	152	33
	Control	144	47	81	30	156	48	67	19	112	36
	Average	177	43	112	30	148	49	106	29	136	38
2	Gas-works liquor	144	27	122	8	115	24	107	15	122	18
	Calcium cyanamide	114	22	87	10	88	20	102	12	98	16
	Calcium chloroacetate	138	12	91	16	130	18	142	11	125	14
	Control	118	22	93	9	139	15	90	10	110	14
	Average	128	21	98	11	118	19	110	12	114	16
3	Gas-works liquor	133	44	135	7	101	27	82	9	113	22
	Calcium cyanamide	129	22	84	16	105	35	74	11	98	21
	Calcium chloroacetate	144	19	93	11	114	22	139	8	122	15
	Control	158	20	141	10	101	14	90	25	122	17
	Average	141	26	113	11	105	24	96	13	114	18
4	Gas-works liquor	155	59	149	18	155	45	154	12	153	34
	Calcium cyanamide	205	32	139	26	154	38	169	5	167	25
	Calcium chloroacetate	263	15	196	12	164	38	189	14	203	20
	Control	187	29	164	11	188	20	124	39	166	25
	Average	202	34	162	17	165	35	159	18	172	26

* 100 lb./plot = 86 cwt./acre.

Table 2. *Treatment trial,* 1939. *Yield (lb./plot)* and viable cyst concentration after cropping (per 10 c.c. soil)*

Centre	Treatment	Plot 1		Plot 2		Plot 3		Plot 4		Average	
		Yield	Cysts	Yield	Cysts	Yield	Cysts	Yield	Cysts	Yield	Cysts
1	Gas-works liquor	140	29	278	46	192	39	125	39	184	38
	Calcium cyanamide	180	24	211	34	290	38	268	44	237	35
	Calcium chloroacetate	182	38	204	29	204	40	227	38	204	36
	Control	263	65	193	56	211	73	223	49	222	60
2	Gas-works liquor	192	39	195	50	182	78	185	54	188	55
	Calcium cyanamide	197	56	216	58	195	78	179	100	197	73
	Calcium chloroacetate	232	46	124	46	212	77	176	56	186	56
	Control	204	92	199	66	179	53	188	46	192	64
3	Gas-works liquor	171	25	111	19	121	14	143	14	136	18
	Calcium cyanamide	155	27	147	25	145	28	177	15	156	24
	Calcium chloroacetate	130	21	137	19	175	16	128	15	142	18
	Control	152	35	143	13	155	8	118	17	142	18
4†	Gas-works liquor	58	23	49	34	58	23	37	33	50	28
	Calcium cyanamide	36	14	38	17	37	18	66	8	44	14
	Calcium chloroacetate	36	16	77	39	16	15	64	17	48	22
	Control	50	20	37	13	72	12	32	27	48	18

* 100 lb./plot = 86 cwt./acre. † Crop lost through summer flooding.

TABLE 3. *Variety trial, 1940. Yield (lb./plot)* and viable cyst concentration after cropping (per 10 c.c. soil)*

Centre	Variety	Plot 1		Plot 2		Plot 3		Plot 4		Average	
		Yield	Cysts	Yield	Cysts	Yield	Cysts	Yield	Cysts	Yield	Cysts
1	Arran Banner	164	26	171	35	172	28	198	21	176	28
	Majestic	188	42	175	55	160	37	226	44	187	44
	Great Scot	157	30	113	30	135	26	137	29	136	29
	Doon Star	195	55	122	38	133	38	161	44	153	44
2	Arran Banner	118	34	188	30	109	36	140	23	139	31
	Majestic	178	48	140	68	169	54	98	58	146	57
	Great Scot	161	38	119	40	132	34	106	25	130	34
	Doon Star	137	43	141	56	158	56	187	47	156	50
3	Arran Banner	160	37	113	33	141	21	130	27	136	30
	Majestic	177	17	165	21	127	28	168	24	159	22
	Great Scot	110	42	125	29	154	40	161	30	138	35
	Doon Star	150	39	174	32	140	15	129	13	148	25
4	Arran Banner	158	32	119	26	105	31	118	19	125	27
	Majestic	126	23	168	63	130	17	94	19	130	30
	Great Scot	123	20	127	70	115	22	96	62	115	44
	Doon Star	113	32	111	14	101	40	110	19	109	26

* 100 lb./plot = 86 cwt./acre.

TABLE 4. *Treatment-variety trial, centre 5, 1939*

Variety	Control					Calcium cyanamide 3 cwt./acre					Calcium cyanamide 2 tons/acre				
	Plot 1	Plot 2	Plot 3	Plot 4	Mean	Plot 1	Plot 2	Plot 3	Plot 4	Mean	Plot 1	Plot 2	Plot 3	Plot 4	Mean
Yield (lb./plot):*															
Arran Banner	16	35	29	11	23	122	99	74	14	77	101	35	44	28	52
Majestic	134	78	94	28	84	42	91	78	67	70	136	84	66	60	86
Great Scot	111	76	55	26	67	85	67	95	44	73	93	72	86	41	73
Doon Star	104	72	65	25	66	49	107	63	55	68	106	110	121	99	109
Viable cysts after crop (per 10 c.c.):															
Arran Banner	11	15	14	16	14	16	13	13	14	14	9	9	13	13	11
Majestic	10	15	15	12	13	8	22	17	16	16	20	17	14	10	15
Great Scot	14	16	21	18	17	13	19	25	16	18	12	8	11	18	12
Doon Star	12	14	21	19	16	12	10	16	24	13	8	12	18	18	14

* 100 lb./plot = 108 cwt./acre.

TABLE 5. *Treatment-variety trial, centre 5, 1940*

Variety	Slaughter-house refuse				Artificial fertilizer				Farmyard manure and artificial fertilizer			
	Plot 1	Plot 2	Plot 3	Mean	Plot 1	Plot 2	Plot 3	Mean	Plot 1	Plot 2	Plot 3	Mean
Yield (lb./plot):*												
Arran Banner	38	58	31	42	74	57	70	67	128	133	120	127
Majestic	83	91	34	69	67	73	60	67	195	179	138	171
Great Scot	42	40	41	41	34	59	30	41	96	129	97	107
Doon Star	45	24	67	45	74	97	42	71	169	116	117	134
Viable cysts after crop (per 10 c.c.):												
Arran Banner	14	17	4	12	26	30	18	25	32	23	42	32
Majestic	8	9	15	11	30	19	12	20	20	23	18	20
Great Scot	19	20	10	16	19	27	14	20	27	35	86	49
Doon Star	6	8	16	10	14	20	15	16	23	14	11	16

* 100 lb./plot = 108 cwt./acre.

Table 6. *Variety trial, centre* 6, 1940. *Yield (lb./plot)* and viable cyst concentration after cropping (per* 10 *c.c. soil)*

Variety	Plot 1		Plot 2		Plot 3		Plot 4		Average	
	Yield	Cysts	Yield	Cysts	Yield	Cysts	Yield	Cysts	Yield	Cysts
Epicure	288	5	280	4	265	<1	246	1	270	3
Arran Pilot	280	1	242	2	277	1	259	2	264	2
Ninety-fold	250	2	272	2	245	1	227	1	248	2
Duke of York	244	3	256	2	237	1	248	2	246	2

* 100 lb./plot = 86 cwt./acre.

Table 7

	Centre	Viable cyst concentration before cropping 10 c.c.	Yield tons/acre	Viable cyst concentration after cropping 10 c.c.
1938	1	34	6	38
	2	7	5	16
	3	8	5	19
	4	4	7½	26
1939	1	9	9¼	43
	2	16	8½	62
	3	19	6¼	20
	4	26	2*	21
1940	1	43	7	36
	2	62	6¼	43
	3	20	6½	28
	4	21	5¼	32
1939	5	5	3¾	15
1940	5	14	4½	21
1940	6	<1	11¼	2

* Crop lost through summer flooding of field.

Field observations

Centres 1–4. In 1938, some of the sets rotted in the ground but the remainder produced satisfactory plants, and except for serious gaps in some Majestic and Doon Star plots differences between the varieties were not marked. Gas-works liquor stimulated growth at all centres and the plants produced the strong, upright stems and dark bluish green foliage associated with heavy nitrogenous dressings. Plants on plots treated with calcium cyanamide also showed the stimulating effects of the nitrogenous dressing, but in a smaller degree than those of plots treated with gas-works liquor. Calcium chloroacetate had no obvious effects on the plants; they were indistinguishable from those on untreated plots. In 1939, similar effects were associated with the treatments though differences were less marked than in 1938. In 1940, little difference between varieties could be detected. There were no signs of potato sickness in any of the crops, though at centre 1 (1940) a patch of small plants occurred. Examination of these plants showed that *Rhizoctonia* was present to a greater extent on this patch than on the rest of the experimental area; this was verified by Dr J. H. Western.

Centre 5. In 1939, plants on all plots were poor and stunted and had yellowish wilting foliage. Plants on plots treated with calcium cyanamide looked better than those on control plots but none were satisfactory. In 1940, plants on plots treated with farmyard manure grew better and looked healthier than plants on other plots.

Centre 6. All varieties at this centre grew well, and conditions for plant growth were so favourable that the trial did not test the response to eelworm invasion and potato-sick conditions.

Notes on yield of potatoes on eelworm-infested land

Throughout these studies the yield of potatoes was used as a measure of plant health and vigour. The summary of yields (which includes seed and chats) given in Table 7 shows that at all centres except centre 6 yields were unsatisfactory. At centre 1 where the field was well cultivated and in a high state of fertility the yield did not exceed 9¼ tons/acre, while at centre 5 (1939) the yield was below 4 tons/acre. The failure of the crop at centre 4 (1939) was associated with summer flooding. During the winter of 1939–40 the drainage system was repaired, but it is probable that some effects of flooding and disturbance were reflected in the low yields of 1940. At centres 2 and 3, where potatoes were grown on the same site for three successive seasons, the yields were higher in the third season than in the first; at centre 2 the yield was highest in the second season, but at centre 3 the increase in yield obtained in the second season was maintained in the third.

Statistical analysis of yield data in Table 1 showed that differences associated with chemical treatment of the soil were significant. Calcium chloroacetate treatment was associated with the highest yields though the substance has no special manurial value. When the treatment trial was repeated in 1939 (Table 2) the results were not consistent with those of 1938. Differences in yield associated with variety of potato were significant in 1938 (Table 1), but these differences were not maintained in other trials (Tables 3–5). At centre 5 applications of calcium cyanamide had no appreciable effect on the yield (Table 4), but a highly significant increase in yield was associated with a dressing of farmyard manure supplemented with artificial fertilizer (Table 5).

At centre 6, where eelworm infestation was negligible, yields averaged 11¼ tons/acre. Statistical analysis of yield data showed that differences associ-

ated with variety were barely significant. As in other trials (Robertson, 1937, 1939) Epicure gave the highest yield, but the yield from Arran Pilot was not significantly lower than that of Epicure.

Influence of initial viable cyst concentration on yield

In field trials hitherto carried out it has been difficult to ascertain and measure the influence of eelworm parasites on yield. Improvement in the condition and fertility of eelworm-infested land was generally associated with increased yields, and since the increased yields were frequently associated with increases in the eelworm population of the soil, the part played by eelworms in limiting the yield has been obscured. A study of Table 7 suggests that the initial viable cyst concentration has some effect on the subsequent yield. The highest yields (centres 4 (1938), 1 (1939), 2 (1939)) were associated with a low or moderate initial viable cyst concentration. It is suggested that the high initial viable cyst concentration at centres 1 (1938, 1940) and 2 (1940) was a factor adversely affecting the yield. At centre 1 (1938) the land was in good heart and potatoes had not been grown for 5 years, and in the absence of eelworms a much higher yield might have been expected. At centres 1 and 2 in 1940 the initial viable cyst concentrations were four times as great as in 1939, and the 1940 yields were markedly lower than those of 1939, while at centre 3 (1939, 1940), which received the same treatment as centres 1 and 2 in 1940, the initial cyst population was approximately the same in both seasons and the 1939 yield level was maintained in 1940.

Influence of yield on cyst production

There is some evidence that yield and cyst production are associated, and this association is most easily seen when the initial viable cyst concentration is low or moderate. Table 7 shows that the greatest increases in viable cyst concentration occurred at centres 4 (1938), 1 (1939) and 2 (1939) where yields were highest. Data from centres 4 (1939), 1 (1940) and 2 (1940) suggest that a high viable cyst concentration is only maintained when the yield is maintained at the level of that obtaining when the original infestation was produced. A fall in yield seems to be associated with a decrease in the viable cyst population, but this should only occur when the cyst population is high and the eelworm population of the host plants is near saturation point.

Effect of treatment on cyst production

It was expected that differences in the number of cysts per plot at the various centres could be associated with treatment since calcium cyanamide and calcium chloroacetate were known to have a deleterious effect on potato-root eelworm. Statistical analysis of data in Table 1 showed that differences in the numbers of cysts associated with treatment were not significant although lowest viable cyst populations were associated with calcium chloroacetate treatment. The treatments were repeated in 1939 (Table 2), but their effects on cyst production were not consistent with those noted the previous year. Again, statistical analysis showed that the differences in numbers of cysts associated with treatment were not significant.

At centre 5 (Table 4) differences in viable cyst concentration associated with treatment were barely significant, but calcium cyanamide at the rate of 2 tons/acre appeared to have some depressing effect on the numbers of cysts formed.

Influence of variety of potato on cyst production

Since such factors as yield, which is associated with variety, also affect the numbers of cysts, it is not easy to isolate the effect of variety on cyst production. Statistical analysis of data concerning numbers of cysts associated with variety showed that differences were not significant except in one series (Table 1) where, as already noted, two varieties had markedly fewer plants than the other two varieties.

The study of an arbitrary cyst/yield ratio, obtained by dividing the total numbers of viable cysts counted by the total yield (in lb.), suggests that the numbers of cysts associated with a unit yield may vary with the variety of potato grown. At centres 1–4 (1938) (Table 1) the cyst/yield ratio was: Great Scot 1·43, Arran Banner 1·14, Doon Star 0·91 and Majestic 0·85. At centre 5 (1940) (Table 5) the cyst/yield ratios are in the same order though the two series are not comparable owing to the difference in the size of the plots: Great Scot 2·18,* Arran Banner 1·75, Doon Star 1·01 and Majestic 1·00. At centre 5 (1939) (Table 4) the cyst/yield ratio was higher for Arran Banner (1·56) than for Great Scot (1·35), but this was caused by an abnormally low yield from one series of Arran Banner plots; the cyst/yield ratios for Doon Star (1·12) and Majestic (1·10) were again low. At centres 1–4 (1940) (Table 3) the cyst/yield ratio for Great Scot (1·63) was again highest, but the order for Arran Banner (1·24), Doon Star (1·54) and Majestic (1·49) was not maintained. These ratios suggest that varietal differences in the host plants may influence either the numbers of eelworm larvae able to invade the roots, the numbers able to survive in the root tissue, or the amount of root tissue available for invasion by eelworms. When considered in relation to the total cyst population of the soil these differences in cyst production associated with host variety may be too small to be of practical importance.

During the three years in which this work has been in progress Mr J. C. F. Fryer, M.A., Director of the Ministry of Agriculture Plant Pathological Laboratory, has taken a keen interest in it and has always been most helpful in discussing procedure. Dr R. Stewart, Adviser in Agricultural Chemistry,

* Plot with abnormally high cyst count was omitted.

Manchester University, has spent much time assisting in the arrangement of the plots and in suggesting methods for the treatment and interpretation of the data. Dr T. Cowling, of Manchester University, has examined data and discussed the relative merits of different methods of interpretation. To these colleagues and to the growers who have at much inconvenience allowed the trials to take place on their farms, the authors express their appreciation and gratitude.

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Observations on the biology of the potato-root eelworm, *Heterodera schachtii* Schmidt

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Results are presented of experiments on the effect of low temperature and of soil type upon the potato-root eelworm, *Heterodera schachtii*.

In some experiments exposure of cysts to low temperatures had a definite lethal effect, but in others the cysts were unharmed. The reason for this variation was not apparent. Exposure of the larvae of the nematode to even a relatively slight degree of frost resulted in the death of the larvae, provided that the surrounding medium was completely frozen. In this respect the larvae appear to differ markedly from those of the beet strain of *H. schachtii*. That the larvae had actually been killed was shown by the fact that they produced no cysts on the roots of potato plants and also by their almost immediate absorption of iodine.

It was confirmed that *H. schachtii* cysts produced on the roots of potato plants grown in a heavy medium are smaller in size and fewer in number than those formed on the roots of plants grown in a light medium. The heavy medium used was pure clay, the light one pure sand, while equal numbers of eelworm larvae served in each case as inoculum. The difference in cyst size was significant, and fewer larvae emerged from the cysts formed in clay than from those produced in sand.

An important disease of the potato plant in this country is caused by the nematode *Heterodera schachtii* Schmidt (*H. rostochiensis* Woll.). The first record of its attack on potatoes in Britain was made by Massee (1913), but 50 years before this another strain of the same eelworm had been recognized as causing severe damage to the sugar-beet crop in Germany. That no suitable remedy other than a long rotation has yet been devised is not due to any lack of attempts to control the nematode by chemical and other means, but perhaps, as Baunacke (1922) has suggested, because our knowledge of the parasite and its various reactions is as yet too incomplete for the vital point of attack to have been discovered. The

aspects of the biology of *H. schachtii* discussed here relate to the effect on the nematode of low temperature and of soil type.

A. The susceptibility of *H. schachtii* to low temperatures

Methods of deep ploughing in winter were used by Karpinski (1902) in an attempt to control the beet strain of the parasite by the exposure of the cysts and of any free larvae in the soil to the action of frost: this did not meet with success. The investigations of Baunacke (1922) suggested that the failure was due to the presence of the parasite at soil levels lower than those penetrated by the plough and also to the ability of the beet strain of the nematode to withstand considerable degrees of frost. Thus, he found that the free larvae of the beet eelworm could withstand repeated freezing at $-8°$ C., and that the embryonic larvae in the egg cases were still alive after having been kept in an ice block at $-9°$ C. for 6 days. Baunacke further claimed that after exposing cysts to a temperature of $-9°$ C. for 6 days their ability to liberate larvae was undiminished. These results were not in agreement with the observations of earlier investigators such as Strubell (1888). The author's experiments concern the effect of frost on the cysts and free larvae of the potato strain of *H. schachtii*.

Experiments with cysts. In the first experiment, (*a*) dry cysts, (*b*) cysts placed in water immediately prior to the exposure to low temperature, and (*c*) cysts previously soaked in water for 6 days were exposed overnight to natural winter frost, the minimum temperature being $-7·2°$ C., while the control cysts were kept at 20° C. In the other experiments cysts treated as in (*a*), (*b*) and (*c*) above were exposed in a refrigerator to temperatures of -5 and $-10°$ C. for short (24 hr.) and longer (7 days) periods. These refrigerator temperatures are the minimum in each case, but there was no reason to suppose that there was much variation in temperature over the whole period. Samples of the cysts were then placed in potato root excretion and the larval emergence from treated and control cysts compared.

In the first experiment the exposure to natural frost resulted in a definite decrease in the subsequent larval emergence, although there was no evidence of mechanical damage to the cysts. It might have been expected that cysts newly placed in water and floating on the surface would have been crushed when the water was converted to ice, or that when cysts which had been thoroughly soaked in water were exposed to frost and the water which they had absorbed had frozen, the cyst wall would have ruptured or else the egg mass would have suffered injury.

The results of the experiments in which cysts were frozen in the refrigerator failed to confirm the injurious effect of low temperature observed in the above experiment, no reduction in larval emergence being detected, even though the cysts were exposed to lower temperatures acting over longer periods. The reason for this discrepancy is not clear.

Experiments with larvae. Suspensions of larvae in potato root excretion were placed in watch-glasses and subjected to both natural and artificially produced low temperatures. The effect of low temperatures was estimated in some experiments by washing the treated and also the control larvae on to pots of sand in each of which a sprouted potato tuber had been planted. In due course, the cysts produced in each pot were extracted and counted. In cases where no cysts were formed it was assumed that the larvae were dead when washed on to the pots. In later experiments Boyd's (1941) iodine-potassium iodide staining method was employed to determine the effect of the low temperatures. By this method the number of dead larvae present can be ascertained more easily, since by applying the stain at a suitable time after treatment dead larvae absorb the stain almost immediately, while living larvae remain for several hours unstained. In all cases observations were made of the number of larvae showing movement before and several days after treatment, in an attempt to provide corroboration of conclusions drawn from the above methods, although it is realized that absence of movement is not a conclusive indication of larval death.

Although, as in the cyst experiments, the temperatures recorded in all cases apart from those of the controls were the minima to which the larvae were subjected, they remained fairly steady throughout, except in the experiment where larvae were frozen outside overnight. Table 1 shows that larvae subjected to overnight frost were killed by this treatment.

Table 1. *Effect of overnight freezing upon potato eelworm larvae*

Total no. of larvae treated	No. of larvae moving before treatment	Min. temp. of treatment ° C.	No. of larvae moving after treatment	No. of cysts produced
96	—	−5·6	0	0
113	—		0	
110	—		1	
118	—		0	
81	32	−10·6	0	0
271	125		0	
100	61		1	
184	109		0	
161	102	Control 20	56	74
169	103		64	
225	134		101	
184	124		97	

The results of the experiments where larvae were frozen in a refrigerator bear out those recorded in Table 1 and are condensed in Table 2.

Table 3 gives the results of experiments in which the iodine-potassium iodide staining test was used

for the recognition of dead larvae. The test was applied 2–5 days after the period of low-temperature treatment. Examination of the control larvae in this way showed that originally about 1 % of these were dead.

These results indicate that the free larvae of the potato strain of *H. schachtii* are more susceptible to the effects of freezing than those of the beet strain (Baunacke, 1922). Baunacke states that exposure to a temperature of −8° C. in ice blocks for 24 hr. had no detrimental effect on the larvae, but in the present experiments most of the larvae of the potato strain were killed after 4 hr. subjection to this temperature. It is, however, impossible to say to what extent differences in technique are responsible for this variation in results.

It was evident in these experiments that potato eelworm larvae could withstand low temperatures where complete freezing of the medium did not take place. Several instances were observed where the larval suspension had frozen completely except for a small part at the base of the watch-glass, and when the medium had been thawed and time given for the larvae to recover many of those in the unfrozen area commenced active movement while those around the edge of the liquid remained motionless and appeared to be dead. Since it was found that the free larvae were killed only when they were completely surrounded by ice, it is possible that the cause of the death of the larvae at low temperatures is the pressure set up by the expansion when water becomes ice.

The lethal influence of frost on larvae of the potato strain of *H. schachtii* is marked and may occur at temperatures which are common during an average British winter. In view of the fact that free larvae can overwinter in the soil (Franklin, 1937) it must be supposed that they do this at a considerable depth, since larvae in the surface layer would probably be killed by severe frost. If there was, therefore, any risk of damage to potato plants by these free larvae it might be diminished by carrying out deep ploughing, thereby allowing the frost to penetrate the soil and act on the larvae of the parasite.

Table 2. *Effect upon potato eelworm larvae of freezing in a refrigerator*

Min. temp. of treatment ° C.	Duration of treatment hr.	No. of larvae treated	Final no. of larvae moving		No. of cysts produced	
			Treated	Control	Treated	Control
−10	24	3400	0	2400	0	325
− 8	48	1500	0	1100	10	159
− 8	6	800	2	400	6	61
− 8	4	1600	5	1100	2	—

B. The effect of soil type on *H. schachtii* cysts

Reinmuth (1929) and Triffitt (1930) recorded that fewer and smaller potato eelworm cysts are found in heavy than in light soils. More detailed experiments were carried out by the present writer, using sand and clay. Duplicate pots of eelworm-free sand and clay, each of which had received 4 g. complete concentrated fertilizer, were inoculated with 10,000 larvae of the potato strain of *H. schachtii* and one Epicure tuber planted in each pot. The control pots received no larvae but were otherwise treated in the same way. It was considered that an inoculum of larvae on two extreme types of soil, by giving a more uniform initial source of infection, would provide more accurate results than those obtained by Triffitt (1930) using an inoculum of cysts, or by Reinmuth (1929) who employed the method of 'lightening' infested heavy soil by the addition of different amounts of sand.

When the growth of the resulting potato plants had ceased and the haulms had died down, the sand and clay from the pots were dried and the newly formed cysts extracted by the Morgan flotation

Table 3. *Effect of freezing upon potato eelworm larvae, determination of the numbers of dead larvae being made by the iodine-potassium iodide staining method*

Total no. of larvae treated	No. of larvae moving before treatment	Treatment ° C.	After treatment		
			No. of larvae moving	No. unstained	No. stained (dead)
82	41	−6·6° for 2 hr.	1	4	78
63	34		0	2	61
53	15	−5° for 15 hr.	2	3	50
47	19		2	2	45
114	54	−3·3° for 5 hr.	3	7	107
121	85		5	9	112
105	61	Control 15°	56	104	1
95	43		33	94	1
152	73		41	151	1
129	75		41	129	0

method. Before extraction the large masses of clay were ground in order to liberate the cysts which they contained. The extracts were dried and sieved and the number of cysts from each pot counted. The cysts from the duplicate pots were then combined and six samples of fifty cysts taken from each set, transferred to potato root excretion and the relative larval emergences counted over a period of 3 months. Measurements of 120 cysts from each soil were made also, using an eyepiece micrometer, and the mean diameters calculated.

Pots containing peat-moss litter were originally included in this experiment but, unfortunately, there was no infection in these pots.

The results are presented in Table 4. No cysts were found in any of the control pots. In the individual pots of each inoculated medium, the numbers of cysts obtained were in close agreement, 952 and 1016 being produced in the sand and 229 and 114 in the clay. The smallest of the cysts measured from the

TABLE 4. *Comparison of cysts produced in sand and clay*

Medium	Av. no. of cysts produced per pot	Av. no. of larvae liberated per cyst	Av. diam. of cysts (mm.)
Sand	984	92·87	0·533 ± 0·0040
Clay	174	63·07	0·478 ± 0·0055

clay had a mean diameter of 0·351 mm. and the largest 0·614 mm. The corresponding figures for cysts from sand were 0·377 and 0·658 mm. The range in larval emergence from samples of fifty cysts produced in sand was from 3353 to 5300 and in those from clay 2195 to 3908. Three facts are clear from the experiment. From an equivalent larval inoculum (*a*) fewer cysts were formed on plants grown in clay than on those in sand, (*b*) the cysts formed in the clay were smaller in size, and (*c*) they produced fewer larvae.

It is hardly likely that the smaller number of cysts formed in the clay was due to such a great invasion of some of the first-formed rootlets that these were killed, thereby causing the death of the larvae in them. If this had been the case, it would have occurred also in the sand, and this hypothesis cannot account for the extreme difference in the numbers of cysts formed in the two media. The smaller numbers of cysts formed in the clay are likely to arise from the difficulty experienced by the larvae and adult male worms in penetrating the dense medium to reach the potato roots, and it is surprising that there was as much penetration as the results indicate. As suggested by Triffitt (1930) lack of aeration in the clay may also play an important part.

The general decrease in size of the cysts produced in clay compared with those formed in sand is most probably due to the increased pressure of the denser medium round the roots of the plant. The plants which were grown in clay were themselves in all respects somewhat smaller than those in sand, as indicated by the yields of tubers which from the control and inoculated plants in sand weighed $5\frac{1}{2}$ and $4\frac{1}{2}$ oz. compared with $3\frac{1}{2}$ and $2\frac{3}{4}$ oz. from the respective plants in clay. It might, therefore, be argued that the smaller plants may not have been able to provide the parasite with sufficient food material with the result that smaller cysts were formed. This view, however, cannot consistently be maintained since relatively large cysts have been observed on small potato plants grown in sand.

It might also be suggested that the main larval invasion of the rootlets in clay took place at a later stage in the life of the host plants than occurred in sand, with the result that most of the cysts formed in clay had not a chance to attain larger dimensions. It is possible that this may have been a contributory factor towards the formation of smaller cysts in the clay.

The experiment also showed that larger numbers of larvae emerged from the larger cysts formed in sand than from the smaller ones produced in clay. This agrees with the relationship between cyst size and egg content shown by Triffitt (1929) and with the relationship between larval emergence and cyst size observed by Gemmell (1940). Whether cyst size in this experiment is determined by the numbers of eggs in the cyst or vice versa appears to depend on whether we regard the variation in cyst size as due to physiological or physical agencies. In other words, if the density of the clay primarily affects the plant whose limited growth tends to reduce the production of eelworm eggs, then this will in turn cause a reduction in cyst size. If, however, the pressure of the clay round the roots of the plant is experienced to any extent by the young cysts, their resultant limitation in size will determine their egg content. Although both lines of action may be involved in certain cases, the evidence of Reinmuth (1929) and Triffitt (1930) suggests that the density of the medium is the primary cause of the reduction in cyst size which results in the formation of fewer eggs and the liberation of fewer larvae.

Liberation of larvae from all these cysts extended over a long period and did not resemble the usual type of 'hatch' in which the rate of emergence, slow at first, rises fairly quickly to a maximum and then decreases more gradually until, after 30–40 days, it is approximately zero. In these observations there were three maxima, at the 8th, 29th and 57th day respectively. This long and unusual type of hatching is thought to be connected with the relatively short period between the formation of the cysts and their exposure to potato root excretion.

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Stimulation of larval emergence in *Heterodera schachtii* Schmidt, by certain concentrations of silver compounds

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Certain dilute solutions of organic silver compounds, e.g. 0·001 % silver proteinate, acting for a short time on cysts of the potato strain of *Heterodera schachtii*, cause increased numbers of larvae to emerge from the cysts when the latter are transferred to potato root excretion. More prolonged exposure of cysts to these solutions tends to result in a toxic effect which is also produced by more concentrated solutions. It is concluded that many more eggs in *H. schachtii* cysts are mature and capable of hatching than can be accounted for by ordinary larval liberation brought about by means of root excretion alone. Increasing periods of pre-treatment of cysts in tap water tend to produce increases in larval emergence, although this stimulation is probably of a different type from that given by silver compounds.

Larvae of the potato strain of the root eelworm *Heterodera schachtii* may be induced to emerge from the resting cysts through the influence of the root excretion of the potato plant. Further, Molz (1932) and Smedley (1936) showed that weak bleaching powder solutions can cause an abnormally great larval emergence both from cysts placed after treatment into potato root excretion and from cysts kept in the original bleaching powder solutions. Following upon field experiments with calcium chloroacetate by O'Brien *et al.* (1939), the unpublished work of two of these authors (Gemmell & Wylie, 1941) showed that this compound also caused increased larval emergence when the treated cysts were transferred to potato root excretion. Hovy (1939) showed that certain organic silver compounds such as silver proteinate could be used as an economic control measure against the tobacco eelworm (*H. marioni*). In the course of unpublished work by the present writer on the action of such compounds on the potato eelworm (*H. schachtii*), it was found that these substances, even in small concentrations, were toxic to the nematode in all stages, but that practical use of the compounds in the control of the disease was uneconomic. With certain very dilute solutions of silver compounds, a stimulation of larval emergence from the cysts was observed, as indicated by the data presented below. The stimulation resembles that already noted by previous workers with respect to calcium chloroacetate and, in part, bleaching powder.

Experimental methods and data

About 1000 potato eelworm cysts, extracted from infested soil a few months previously, were transferred into each of a series of watch-glasses placed on moist filter paper in large Petri plates. Into each of the watch-glasses was introduced 2 c.c. of the required concentration of either a silver proteinate, benzoate, or lactate solution. The strengths of these solutions ranged from 0·05 to 0·001 % and the cysts were kept immersed for periods up to 6 weeks, at a constant temperature of 20° C. After 1 day, and 1, 3 and 6

weeks respectively, three sets of fifty cysts were extracted from each watch-glass, washed in drops of water and then placed in 2 c.c. of potato root excretion in clean watch-glasses and kept at 20° C. Cysts pre-treated in tap water were used for comparative purposes.

The larval emergences were counted periodically by means of a binocular microscope until hatching had for all practical purposes ceased. At intervals during the hatching process the root excretion was replaced by a fresh quantity.

The cysts were then stored in a dry condition for 48 days, replaced in root excretion, and the further 'hatches' counted. The numbers of larvae liberated in this 're-hatch', although much smaller than in the first case, tended to be in the same proportion as the corresponding numbers in the primary hatch. The total numbers of larvae liberated in the two hatching periods were expressed as percentages of the controls (Table 1).

Table 1. *Total larval emergences from cysts immersed for different periods in solutions of silver compounds*

Silver compound	Conc. of sols. %	Conc. of sols. Mg. silver/l.	Larval emergences (% of control) 1 day	1 week	3 weeks	6 weeks
Proteinate	0·05	40	5	0	0	2
	0·01	8	64	47	19	39
	0·005	4	119	99	87	28
	0·001	0·8	302	192	108	113
Benzoate	0·05	292	0	0	0	0
	0·01	58·4	1	0	0	0
	0·005	29·2	112	13	2	4
	0·001	5·8	201	179	119	106
Lactate	0·05	274	0	0	0	0
	0·01	54·8	4	2	0	0
	0·005	27·4	209	36	5	1
	0·001	5·5	187	54	55	51
Control (water)	—	—	100	100	100	100
actual no. of larvae			(2882)	(3451)	(3552)	(3862)

Table 1 indicates that stronger solutions of the silver compounds prevented or reduced hatching: weaker solutions (0·001–0·005 %) promoted hatching, at least initially. The effect of the various concentrations of the different compounds does not appear to be governed solely by their silver content. It is particularly to the stimulatory effect that attention is drawn. This stimulation was especially evident with a silver proteinate solution containing 0·8 mg. silver/l., which after 1 day induced an emergence three times greater than that of the control. When cysts were transferred to potato root excretion after 1 week's immersion in this proteinate solution, hatching again exceeded the control, although to a reduced extent. After 3 weeks' immersion the subsequent output of larvae was practically at the control level.

The increase in emergence caused by such solutions as 0·005 % silver proteinate and 0·001 % silver lactate gave place later to a tendency to inhibit hatching, indicated by the relative figures after 6 weeks' treatment (see Table 1). This may have been due to a cumulative poisoning of the cyst contents having been produced by the longer time of action of these solutions. Solutions such as 0·001 % silver proteinate and 0·001 % silver benzoate which also had an early stimulative action had no toxic effect even after 6 weeks' immersion, when their larval emergences were closely comparable to that of the control. There must be a time between the stimulative and toxic effects of some of these solutions when they will act in the same way as water, and it may be that the two last-mentioned solutions would, if allowed to act for longer than 6 weeks, prove toxic to the cyst contents. On examination of the total numbers of larvae which emerged from the control cysts, previously immersed in water for various lengths of time, a steady increase is evident corresponding to increase in time of storage. From this it would appear that water itself has a stimulative effect on the eggs contained in eelworm cysts.

A further experiment using silver proteinate alone on cysts extracted from an infested field 2 years previously gave comparable results as regards stimulation, the observed increases being greatest after only 12 hr. immersion in a 0·001 % solution. All the 'hatches' in this experiment were much smaller than those in the first experiment, due to the fact that older cysts with a smaller viable egg content were used. Also, these cysts were slightly more susceptible to the action of the silver solutions than those formed more recently, possibly because a breakdown of part of the contents of the older cysts allowed the solutions to penetrate more quickly and more deeply. This experiment also confirmed the indication that water has a stimulative effect on the cyst contents.

Discussion

The above results reveal that certain dilute concentrations of the organic silver compounds employed had a stimulative effect on the contents of potato eelworm cysts immersed in them for periods of about

24 hr. This effect was maintained even when the cysts were 're-hatched' after being stored in a dry condition.

That the dilute silver solutions do not act like potato root excretion and cause larvae to emerge directly when cysts are kept in the solutions was shown by the fact that the small numbers of larvae liberated into these solutions were similar to the numbers which emerged into the tap water.

Calcium chloroacetate and bleaching powder have also the property of causing the liberation of an increased number of larvae, and the latter compound brings about larval emergence without the presence of root excretion. Molz (1932) attributed the increased rate of hatching to a physiological stimulus, believing it to be due to the liberation of free oxygen from chloride of lime and to the alkaline nature of the compound. Smedley (1936), however, was of the opinion that the stimulative power of the hypochlorites is mainly the result of their peculiar action on proteins and that hypochlorite solutions containing calcium attack the egg membranes in the eelworm cysts, inducing the eggs to hatch without the presence of root excretion.

The stimulative power of the silver solutions may also be due to their effect on the proteins, since in cases where silver exerts a toxic influence on protoplasm the principal reaction, according to Seifriz & Uraguchi (1941), probably takes place between the silver and the protoplasmic protein. The net result of the action of the dilute silver solutions is to prepare the eggs so that the root excretion can exert its maximum effect on the cyst contents and thus set free many more larvae than would otherwise be the case had the cysts been pre-treated with tap water. It might be suggested that the stimulative action of these solutions causes immature eggs to mature before the root excretion begins to take effect. If this be so, maturation must occur very quickly so that the eggs are immediately ready to hatch after only a brief contact with the stimulating solution, for not only did larval emergence from stimulated and control cysts commence and finish practically simultaneously but the graphs of their progress reached their maxima at the same time. In view of these facts it is hardly likely that the effect of the silver solutions is to bring about maturation of the eggs.

A more satisfactory view is that many more of the eggs in a cyst are ready for hatching than can be accounted for by the number of larvae which normally emerge when the cyst is placed directly into root excretion. If this be so, then in the cysts used—apart from any immature eggs which may or may not have been present—there were mature eggs which hatched under the influence of root excretion alone and also mature eggs which hatched only after pretreatment with a dilute solution of a silver compound. This variation in hatching agrees with the known fact that all the eggs contained in potato eelworm cysts do not liberate their larvae during the first year in which the cysts are in contact with the excretion from potato roots. Many of the eggs remain unhatched in the cysts and serve to carry over the potential infection for at least 2 years. This happens under field conditions. In laboratory experiments, so far as the writer is aware, no potato root excretion has yet been produced which will cause all the eggs in newly formed cysts to hatch during their first period of contact with root excretion, although Triffitt (1930) indicated that all eggs in overwintered cysts are capable of hatching under favourable conditions in the season following their formation. It may be that in the cysts there are layers of eggs to which the root excretion cannot immediately penetrate, but as yet the mechanism which prevents apparently mature eggs from hatching is not definitely known.

Evidence that there is an increase in the output of larvae from cysts pre-treated for increasing lengths of time in tap water was observed in these experiments. This is not stimulation of the type shown by silver compounds or by hypochlorites, but is probably the result of absorption of water which encourages and influences the development and maturation of eggs within the cysts.

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Field experiments on the control of sheep maggots

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(With 3 Text-figures)

Field experiments on about 4700 sheep at about forty different farms in North Wales are discussed. Crutching is the most effective control measure for lambs. Regular dipping with commercial arsenic-sulphur dips gives reasonable control except during adverse weather conditions. Dipping with zinc arsenite or with calomel proved, on the whole, less effective than dipping with commercial dip. Addition of calomel to commercial dip proved to be more effective than commercial dip alone. Preliminary observations are presented on the variation of the surface tension and viscosity of the dipping fluid as the number of sheep passing through the dip increased.

In his reports on the sheep-maggot problem the late Dr R. P. Hobson (1941) drew attention to the principal factors which create conditions most suitable for infestation, reviewed existing methods of control by physical and chemical means, and indicated promising new methods by which the variety of problems arising from maggot infestation might be tackled. The results now presented are the outcome of about forty field tests which were made with approximately 4700 sheep at different farms in the four counties of North Wales during the summer of 1941 with the object of extending the field work of Dr Hobson. Essentially the investigations fall into five categories: (*a*) experiments on crutching; (*b*) experiments on dipping with zinc arsenite; (*c*) experiments on dipping in a suspension of calomel; (*d*) experiments on dipping in a mixture of arsenical dip and calomel; (*e*) collateral investigations of factors affecting strike.

Crutching

Suggestions that crutching might be carried out on a flock never found ready acceptance by farmers because of a deep-seated conservatism with regard to the disfigurement of lambs and the probable misapprehension by the buyer that a crutched lamb had been infested with maggots. These doubts are not well founded. After about a month the crutched lamb is barely distinguishable from the uncrutched; and 5 weeks after crutching, a crutched and an uncrutched lamb can be distinguished only by examination. The results (Table 1) cover a period of 43 days for each experiment. Grazing conditions, treatment of control (i.e. uncrutched) flock, sex, breed, shepherding and other factors introduce complications in interpretation of the data which are noted in the final column of the table.

Table 1. *Effect of crutching*

	Crutched flock			Control flock (not crutched)			
		No. struck			No. struck		
Exp.	No. of lambs	On tail	On body	No. of lambs	On tail	On body	Remarks
1	30	—	1	30	17	—	Neither flock dipped. Poor pasture
4	22	1	2	32	12	1	Neither flock dipped. Shortage of pasture
7	30	—	—	42	—	—	Control flock dipped in arsenic-sulphur dip. Locality comparatively free from strike throughout the whole summer
10	12	1	—	12	1	2	Crutching not sufficiently close. Neither flock dipped. Excellent shepherding. Ewe lambs only
16	8	1	—	25	2	—	Control flock dipped very efficiently
23	15	—	—	20	—	—	Neither flock dipped. Grazing on bracken-infested upland pasture (Ffriddland)
Totals	117	3	3	161	32	3	
		6			35		
% of total		5·1			21·8		

The data in Table 1 show that the probability of strike in an uncrutched lamb is approximately *four* times greater than in a crutched lamb. Considering tail strikes only, the most common form in lambs, the probability of strike is *eight* times greater in uncrutched than in crutched lambs.

These data show that very considerable protection against strike results from crutching, and receive confirmation from the published results of Hob-

son and the universal practice of crutching in Australia.

DIPPING WITH ZINC ARSENITE

The results are summarized in Table 2, and strikes for each experiment are tabulated for the first 34 days after dipping. For all the dipping experiments 'control' refers to sheep dipped in a full-strength arsenic-sulphur dip.

not only of strikes but also of restrikes. Of the sixty 'test' lambs in Exp. 35 four were struck four times and quite a number two or three times. None of the 'control' lambs was struck more than twice and the number struck twice was much less than the re-infested 'test' lambs. It is, therefore, concluded that zinc arsenite has, apparently, no ovicidal action and that its larvicidal action is not better than, even if as good as, that of a commercial arsenical dip.

TABLE 2. *Trials with zinc arsenite dip*

	Zinc arsenite dip							Commercial arsenical dip						
	Conc. of zinc arsenite as As_2O_3	No. of			No. struck			Conc. of As_2O_3	No. of			No. struck			
Exp.	%	Ewes	Year-lings	Lambs	Ewes	Year-lings	Lambs	%	Ewes	Year-lings	Lambs	Ewes	Year-lings	Lambs	Remarks
12	0·2	—	—	47	—	—	2	0·2	—	—	26	—	—	4	Dipped on same day in same dip
13	0·2	—	—	38	—	—	4	0·2	—	—	30	—	—	5	Dipped on same day in same dip
17	0·2	32	—	13	1	—	1	0·2	43	—	25	1	—	1	Dipping very efficient
20	0·1	—	14	50	—	2	0	0·2	—	14	50	—	2	4	
24	0·15	151	—	124	—	—	3	0·2	154	—	143	—	—	3	Good " dipping; grazing in wooded, bracken-covered park
35	0·2	—	—	60	—	—	55	0·2	—	—	79	—	—	43	Heavy rain 3 hr. after dipping. Bad weather
36	0·2	173	—	79	9	—	24	0·2	112	—	—	17	—	—	Dipping on same day. Flocks grazing on different pasture. See Fig. 1
Totals		356	14	411	10	2	89		309	14	353	18	2	60	
% struck		—	—	—	2·8	14·3	21·6		—	—	—	5·8	14·3	17·0	
Gross totals		781			101				676			80			
Over-all % struck		12·9							11·8						

TABLE 3. *Trials with calomel dip*

	Calomel dip					Commercial arsenical dip					
	Conc. of calomel	No. of		No. struck		Conc. of As_2O_3	No. of		No. struck		
Exp.	%	Ewes	Lambs	Ewes	Lambs	%	Ewes	Lambs	Ewes	Lambs	Remarks
5	0·02	—	29	—	3	0·2	—	30	—	5	Good dipping. Sheep rather heated at time of dipping
6	0·02	—	30	—	2	0·2	—	30	—	0	Good dipping
8	0·015	57	24	0	0	0·08	39	72	0	0	—
11	0·10	69	49	1	2	0·2	80	71	3	7	Grazing changed after 36 days, with consequent increase in maggoting
26	0·2	52	35	8	1	0·2	45	40	3	3	—
27	0·2	32	21	0	0	0·2	26	24	0	4	Dipped immediately after those of Exp. 26. Flocks of Exp. 26 were grazing near the sea-shore; those of Exp. 27 about 1 mile inland
29	0·175	—	57	—	16	0·2	—	57	—	13	See Fig. 2
Total		210	245	9	24		190	324	6	32	
% struck				4·3	9·8				3·2	9·9	
Gross totals		455		33			514		38		
Over-all % struck		7·3					7·4				

Table 2 shows that zinc arsenite is only about 80 % as effective as commercial dip in protecting lambs. The apparently better protection given to ewes may be entirely fortuitous but is a point which merits fuller investigation. The shepherd who recorded the strikes for Exps. 35 and 36 kept very detailed records

DIPPING WITH CALOMEL

Seven experiments were carried out using calomel as the dipping agent. The results, summarized in Table 3, extend over 39 days in each experiment.

Combined with Hobson's data (1941 *c*), these re-

sults do not lead to recommending calomel instead of commercial arsenic-sulphur dips. Also, there are other factors which militate against its use, viz. its much higher price, its lack of colour, the difficulty of preparing and maintaining it in suspension, and the fact that it does not kill keds or ticks.

Farmers were more ready to allow this experiment than dipping with calomel or zinc arsenite alone, probably for two reasons, (i) that it could be carried out without emptying the bath, (ii) the dipping fluid was coloured. The fact that the bath was not emptied means that all test flocks discussed in this section

TABLE 4. *Trials with a mixture of commercial arsenical dip and calomel*

	Mixture					Commercial				
	% calomel	No. of		No. struck		Conc. of As_2O_3 %	No. of		No struck		
Exp.	added	Ewes	Lambs	Ewes	Lambs		Ewes	Lambs	Ewes	Lambs	Remarks
						(*a*) Duration 52 days					
2	0·01	—	50	—	1	0·2	—	50	—	3	Good dipping; 146 lambs dipped before calomel added
3	0·015	—	53	—	9	0·2	—	53	—	25	Good dipping; about 150 sheep dipped before calomel added. Incidence curve, Fig. 3
15	0·1	37	17	0	2	0·2	36	16	2	1	Dipping good but pens dirty
						(*b*) Duration 31 days					
9	0·02	15	25	0	2	0·1	28	16	0	0	Dipping conditions not very good. Weather unsuitable for strikes
31	0·1	31	16	3	3	0·2	23	17	0	8	Commercial dip contained a wetting agent
34	0·05	—	168	—	6	0·2	—	160	—	16	Both flocks comprised wether lambs. Control flock dipped after 645 ewes and ewe lambs had been through bath. Then calomel added (i.e. after 805 sheep had passed through bath) and test flock dipped. Dip made up from 3 parts commercial arsenical dip and 1 part zinc arsenite
37	0·1	107	29	31	12	0·2	112	79	16	40	Commercial dip contained a wetting agent. Incidence curve, Fig. 4
38	0·05	—	99	—	7	0·2	—	82	—	4	Both flocks comprised wether lambs
Totals		190	457	34	42		199	473	18	97	
% struck				17·9	9·2				9·0	20·4	
Gross totals		647		76			672		115		
Over all % struck				11·7					17·1		

DIPPING WITH CALOMEL ADDED TO COMMERCIAL ARSENICAL DIP

Hobson (1941) advocated the addition of an ovicide to commercial dips but considered that calomel was unsuitable. He does not record having tested out this hypothesis.

The mechanism of the ovicidal action of calomel is not fully understood. Therefore, it was felt that experiments incorporating calomel in an arsenic-sulphur dip were worth trying, although it was realized that calomel would be decomposed by the dip. On addition of calomel the dip darkens in colour and probably finely divided mercury is formed as one of the resultant products.

were dipped in the bath *after* the control flocks had been dipped. Therefore, the test flock always passed through a fouled bath. This fact is of supreme importance in assessing, from the experimental data, the suitability of the addition of calomel to a commercial dip as a preventive measure.

Thirteen experiments were carried out under a variety of different conditions of grazing, climate, duration of test and amount of calomel added to the dip. The results are summarized in Tables 4–6. The data in Table 4 (*a*) cover a period of 52 days, those in Table 4 (*b*) 31 days, and those in Table 5 38 days, but separate records for strikes in ewes and in lambs were not maintained in the three experiments reported in Table 5. Table 6 has been compounded

from Tables 4 and 5. Where only part of the flock was used, suitably marked, for the experiment, the number of sheep which had passed through the bath before the addition of calomel is given in the appro-

tion of Tables 4–6 and Figs. 1 and 2. Addition of calomel to commercial dips (various brands were used) leads to a distinct improvement and, for lambs, there is markedly greater protection against

TABLE 5. *Trials with a mixture of commercial arsenical dip and calomel (duration 38 days)*

	Mixture				Commercial				
	Calomel added	No. dipped		No.	Conc. of As_2O_3	No. dipped		No.	
Exp.	%	Ewes	Lambs	struck	%	Ewes	Lambs	struck	Remarks
14	0·1	73	12	1	0·13	63	18	7	Good dipping conditions
30	0·1	105	37	17	0·2	78	71	7	Records insufficiently detailed to draw incidence curve
33	0·17	82	39	25	0·13	83	40	27	Control flock dipped; bath emptied, new dip made up for test flock
Totals		260	88	43 (12·4%)		224	129	41 (11·6%)	
		348				353			

TABLE 6

(Compounded from Tables 4 and 5)

	No. dipped		No. struck		% struck		No. of control flock struck per strike in test flock
	Test	Control	Test	Control	Test	Control	
Ewes	190	199	34	18	17·9	9·0	0·50
Lambs	457	473	42	97	9·2	20·4	2·58
*Total	647	672	76	115	11·7	17·1	1·46
†Total	995	1025	119	156	11·7	15·2	1·29

* Excluding data in Table 5. † Including data in Table 5.

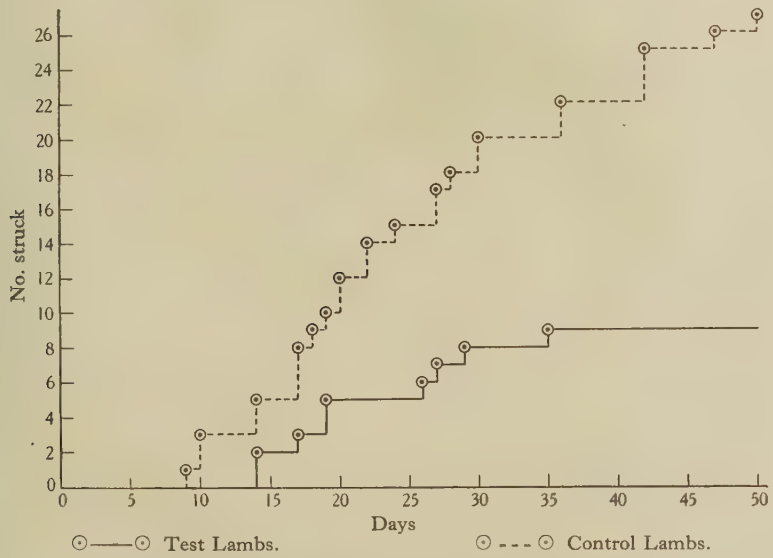

Fig. 1. Incidence of strike in flocks dipped in commercial arsenical dip (0·2% As_2O_3) + calomel (0·015%) (test flock) and in commercial dip alone (0·2% As_2O_3) (control flock). Exp. 3.

priate table; otherwise, the entire control flock was dipped, calomel was added, and the test flock then dipped.

Several interesting points arise from an examina-

strike when calomel is added. For ewes, on the other hand, the results (Table 6) are at first sight disappointing. Reference to Table 4 shows that all the ewes which became infested had been dipped

in a commercial dip containing a wetting agent and thereby much more easily mixed. The reason for this heavy infestation is probably that the wetting agent in the dip causes more complete drainage from the fleece. Ewes, at the time of dipping, have much shorter fleeces than lambs, and, therefore, the active mercurial principle is retained to a much less extent in the fleece of the ewe than in the fleece of the lamb.

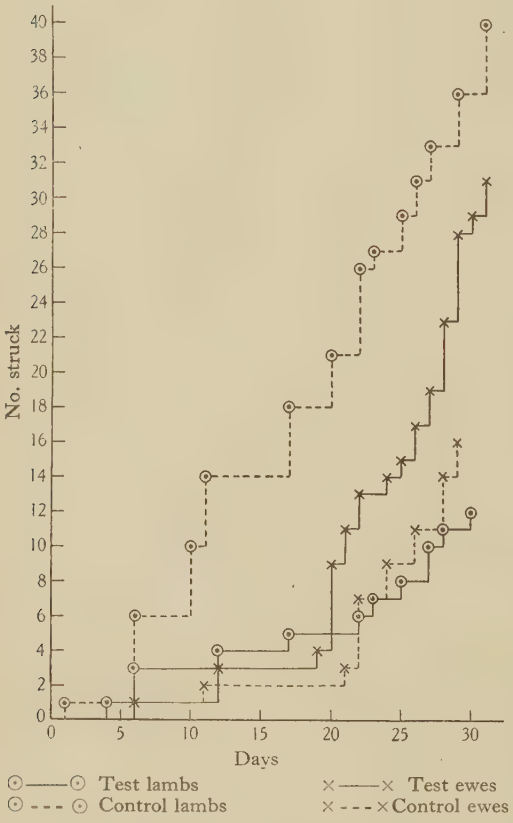

Fig. 2. Incidence of strike in flocks dipped in commercial arsenical dip (0·2 % As_2O_3) + wetting agent + calomel (0·1 %) (test flock) and in commercial dip + wetting agent (0·2 % As_2O_3) (control flock). Exp. 37.

If this active principle is *finely divided* mercury (as it most probably is) or some salt of mercury, these finely divided particles would be less readily retained than the macroscopic particles of the commercial dip as the dipping fluid filters its way out of the fleece during drainage.

Although from this one series of experiments definite conclusions cannot be drawn, the improvement produced by the addition of calomel does not seem to be related to the concentration of calomel used. In fact, when small concentrations of calomel are used, the results favour the admixture (vide Exp. 2, Table 4), whilst larger concentrations in some experiments gave less favourable results (vide Exp. 33, Table 5). This raises two questions: (i) is there an optimum concentration of calomel, and (ii) is this due to an incomplete reaction between calomel and the dip when 'excess' calomel is used, leading to an equilibrium condition being established between calomel and the products of its reaction with the dip? With regard to (i) an answer can only be given after further field investigations. The second question could be answered only after thorough investigation of the chemical reaction which occurs.

General discussion of dipping experiments

In presenting the data ascertained from the dipping experiments, detailed discussion of each experiment has not been given because the experiments were carried out over a wide area, on different breeds of sheep grazing on a variety of pastures from rough mountain to sandy sea-shore cliffs. Weather conditions varied with regard to rainfall, atmospheric pressure and temperature, and relative humidity. Therefore, no basis for comparison between individual experiments of the same type exists.

Dipping procedure, too, varied. Most baths were good, including 'home-made' baths, although these invariably had been constructed with no drainage and consequently had to be emptied by hand. Later, preliminary data are given for the variation of physical properties of the bath with the number of sheep dipped, and these, suitably extended, may lead to the desirability of being able to empty the bath frequently and quickly. The capacity of many home-made baths had not been accurately measured, and it is urged that attention should be given to this so that the best results may be obtained from dipping. Explicit instructions are given regarding the amounts of commercial dips which must be used to give the best results: these can only be carried out if the capacity of the bath is known.

Catching pens were frequently very dirty and fleeces get soiled prior to dipping, with the result that the dip became unnecessarily fouled. Fouling of the bath occurs from two other causes: (*a*) from animals defaecating and urinating as a result of shock and excitement when they are immersed in the dip, and (*b*) from drainage fluid returning dirty from the dripping pens. Every care should be taken to minimize the amount of fouling, and on many farms improved catching pens and more frequent sweeping of dripping pens would lead to better results. The frequent skimming of the greasy scum from the surface of the bath is necessary to prevent its becoming entangled in the fleece.

Several other points in dipping procedure were noted. Rough handling of sheep, although infrequently encountered, was traceable to inexperienced labour or, more often, to catching pens

being too large. Considerable variation in the extent and times of immersion existed. In some instances the dip was not well mixed and a proper suspension was not formed. In most cases farmers and shepherds had a strong, sometimes impenetrable, prejudice against a colourless dip because it gave no 'bloom'.

In these investigations no purposeful attempt was made to correlate incidence of strike with grazing conditions. Lowland flocks were most liable to strike, particularly those grazing on pastures or sandy belts near the sea. If pastures were overstocked and close-cropped, maggoting was more prevalent, probably on account of fleeces becoming soiled when the sheep lie down. More comparative data on different breeds would be very desirable. One such set of data were obtained in Exp. 29 (duration 44 days), where the test flock was dipped in calomel and the control flock in arsenic-sulphur dip. Both flocks comprised thirty-four Welsh and twenty-three Welsh-Southdown lambs. Strikes occurred as follows (see also Fig. 3):

Test		Control	
Welsh	Cross	Welsh	Cross
8 (23·6 %)	12 (52·2 %)	17 (50 %)	2 (8·7 %)

No explanation of these data can be offered, but their interest is undeniable.

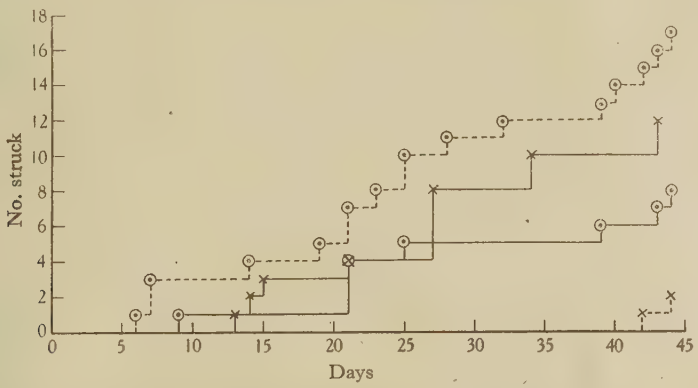

Fig. 3. Incidence of strike in Welsh and Welsh × Southdown lambs dipped in calomel (0·175 %) (test flock) and in commercial arsenical dip (0·2 % As_2O_3) (control flock).

Physical properties of the dipping bath

Following the work of Hobson (1939, 1941*d*) on the effect of suint on sheep dips, a few preliminary measurements of the viscosity and surface tension of the bath were made to ascertain to what extent these varied as the number of sheep dipped increased and, therefore, suint and fouling increased. Samples were filtered and surface tension was measured by the Traube stalagmometer method and viscosity by an Ostwald viscometer. Viscosity increased almost linearly with the number dipped, but the rate of increase varied from farm to farm. Surface tension decreased almost linearly, passed through a minimum, and then increased. Again, rates of decrease were specific, but it is noteworthy that the greater the increase in viscosity, the greater the initial decrease in surface tension. These puzzling results probably arise from variations in the natural properties of the water supplies, particularly the hardness of the water. Conclusions cannot be drawn because of the paucity of data, but available results point to the desirability of a more detailed and extended investigation.

It is a great pleasure to record our thanks to the farmers for their enthusiastic co-operation and kindly welcomes; to the Agricultural Research Council for a grant; and to Prof. R. G. White for his interest, encouragement, and help.

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Studies in the mechanism of fungicidal action

V. Non-metallic and sodium dithiocarbamic acid derivatives

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(With 4 Text-figures)

This investigation has as its object the elucidation of the mode of action of dithiocarbamic acid derivatives as fungicides, with the help of the theory of variability, heavy metal salts being dealt with separately.

The experiments were carried out using *Macrosporium sarcinaeforme* as the test organism; the chemical materials used are listed.

Deviations from the experimental and mathematical techniques described in previous papers of this series are explained.

From the results it is inferred that: (*a*) dithiocarbamates are decomposed by the spores to a greater or lesser degree into amine and carbon disulphide, both being toxic; (*b*) the amines can be absorbed only in a combined form, which may be the dithiocarbamate itself but is more probably a derivative, possibly an ester; (*c*) the carbon disulphide probably acts through the mediation of a thiocarbonate; (*d*) thiuram-sulphides act through being decomposed to dithiocarbamates (or their derivatives mentioned above). The monosulphides are largely, and the disulphides slightly, decomposed; in the latter case with reduction.

It is suggested that the greater toxicity of the methyl-derivatives over all others tested is due to the shortness of the hydrocarbon chain.

It is concluded that the role of the carbon disulphide is chiefly that of a chemical carrier of the amino group, but that, owing to the negative index of variation of the spores to the latter, the carbon disulphide must play an essential part in completing the toxicity.

It is suggested that heavy-metal dimethyldithiocarbamates may show promise against *M. sarcinaeforme*, but that others may be effective against other fungi.

I. Introduction

The importance of developing fungicides and insecticides from non-imported sources made it desirable to explore the fungicidal possibilities and mode of action of the dithiocarbamates and thiuram-sulphides. These compounds are prepared commercially for use in the processing of rubber, and a supply of derivatives of dithiocarbamic acid is thus available. That these substances might find wider use as fungicides is suggested by several references in the literature.

Goldsworthy *et al.* (1942) found in laboratory and field tests that the derivatives of dimethylamine were the most toxic, and of various salts which they investigated considered that plumbous dimethyldithiocarbamate was the most promising. Palmiter & Hamilton (1942) also reported favourably on heavy metal dithiocarbamates. Tisdale & Flenner (1942) found that of all these compounds sodium dimethyldithiocarbamate was the most toxic to the chlamydospores of *Ustilago hordei*; it was highly effective at 33 p.p.m. Tetramethylthiuram-monosulphide was effective at twice this concentration; these workers reported that toxicity is inversely related to stability among the metallic salts. They reported many cases of fungicidal effectiveness among these compounds demonstrated in the field, the best being ferric dimethyldithiocarbamate, especially when mixed with calcium hydroxide, which was effective against *Coccomyces hiemalis* on rose and *Venturia inaequalis* on apple. It is reported by Guy (1937) that the thiuram-sulphides, especially tetramethylthiuram-disulphide, are useful as repellents of insects, e.g. Japanese beetle.

In view of this work the following aims were set for the present investigation: (i) to ascertain the reason for the reported superiority of the dimethyl derivatives, if possible in terms of their electronic structure; (ii) to elucidate the role of the sulphur-containing part of the molecule; (iii) to suggest in what form the fungicidal properties of the dithiocarbamate derivatives could best be utilized in practice.

II. Experimental methods

Biological material

Experiments were carried out using *Macrosporium sarcinaeforme* as the test organism. The cultures used were direct descendants of those used in previous work (Parker-Rhodes, 1941 *et seq.*) and were maintained and used by the technique there described. Owing, however, to a spontaneous loss of sporulation in the strain used, a fresh strain was used for the last experiments, obtained by monospore isolation from

the old one. The isolate selected responded to the toxic action of copper sulphate like the original strain; it had vigorously growing germ tubes, and did not show fragility of the conidiophores, two factors which facilitate germination counts.

Chemical material

The following substances were employed in the experiments:

(i) Sodium dimethyldithiocarbamate: $(CH_3)_2NCSSNa$.
(ii) Sodium diethyldithiocarbamate: $(C_2H_5)_2NCSSNa$.
(iii) Sodium *diiso*propyldithiocarbamate: $((CH_3)_2CH)_2NCSSNa$.
(iv) Sodium dibutyldithiocarbamate: $(C_4H_9)_2NCSSNa$.
(v) Sodium pentamethylenedithiocarbamate: $C_5H_{10}NCSSNa$.
(vi) Sodium ethylphenyldithiocarbamate: $C_6H_5C_2H_5NCSSNa$.
(vii) Dimethylammonium dimethyldithiocarbamate: $(CH_3)_2NCSSN(CH_3)_2H_2$.
(viii) Dimethylammonium chloride: $(CH_3)_2NH_2.Cl$.
(ix) Piperidinium pentamethylenedithiocarbamate: $C_5H_{10}NCSSC_5H_{10}NH_2$.
(x) Tetramethylthiuram-monosulphide: $(CH_3)_2NCS.S.CSN(CH_3)_2$.
(xi) Tetramethylthiuram-disulphide: $(CH_3)_2NCS.S_2.CSN(CH_3)_2$.
(xii) Dipentamethylenethiuram-monosulphide: $C_5H_{10}NCS.S.CSNC_5H_{10}$.
(xiii) Dipentamethylenethiuram-disulphide: $C_5H_{10}NCS.S_2.CSNC_5H_{10}$.

Specimens of all these were kindly supplied from the laboratories of Messrs Robinsons, Ltd., of Birmingham, who also supplied specimens of the amines corresponding to (i), (ii), (iv), (v) and (vi). Nos. (vii) and (viii) were prepared by the action of the calculated amounts of HCl on (i) followed by expulsion of the liberated CS_2 in a current of nitrogen. Nos. (x)–(xiii) were used in aqueous suspension, the others in solution.

Technique of toxicity tests

In general the methods used were the same as those previously described (Parker-Rhodes, 1941); the following features were new:

In counting germination increased accuracy was obtained by counting 100 spores in each test drop instead of 50. Furthermore, acting on the observation of McCallan *et al.* (1941) that identical replicate drops behave statistically as equivalent random samples of the given population, the count was made on 500 spores wherever possible, even if one of the drops was lost or disturbed, by counting 200 spores in one of the remaining drops: 600 spores were counted on sprayed slides.

Considerable difficulty was experienced in making suspensions of the thiuram-sulphides fine and stable enough to pass evenly through the atomizer used in spraying. Stabilizing colloids were found to give fairly satisfactory suspensions, when, to a dilute solution of the colloid, was added a concentrated alcoholic solution of tetramethylthiuram-monosulphide. But the suspensions thus obtained were too dilute to be sufficiently toxic, and the following method was used. The thiuram-sulphide was triturated in a mortar with about 5 times its weight of bentonite to a fine powder, water was added little by little, the paste being worked to homogeneity each time, until fluid: it was then made up to the required volume. The chief defect of this method lay in the difficulty of making up a quantitative suspension due to the stickiness of the material.

III. MATHEMATICAL METHODS

The results were analysed by the methods of the theory of variability as described and exemplified in previous papers of this series. Certain novel features were encountered in this work which call for comment.

Estimation of significance

Parker-Rhodes (1942*c*) drew attention to a difficulty arising in applying the methods of Bliss (1936*a*, *b*) to the analysis of fungicidal data due to the unknown degree of interaction of the spores in the experimental drops. It was proposed to denote by D the number of spores forming each of those units which could be considered as acting independently, and a method of estimating D was suggested. Unfortunately, this method requires a very large body of experimental data, and subsequent experience has shown that D has tended to fall (presumably owing to some change in the fungus culture used). On this account recourse was had, on the advice of Mr D. J. Finney, to the admittedly false assumption that $D = 1$. The χ^2-values being therefore always significantly greater than expectation, the statistics were compared by means of the t-test (Bliss, 1936*b*). The results by this method did not differ qualitatively from those obtained assuming the best (recent) estimate of D and using the χ^2-test of significance. Using the notation of the theory of variability, the formula for t_b is

$$t_b = \sqrt{\left(\frac{U_1 + U_2 - 2\sqrt{(U_1U_2)}}{U_1U_2\left(\frac{\chi_1^2}{n_1}\alpha_1 M_1 + \frac{\chi_2^2}{n_2}\alpha_2 M_2\right)}\right)}.$$

Modal tolerance and median lethal dose

It has been reported that under the conditions of these experiments the modal tolerance of the spores (m) is liable to fluctuate from one experiment to another on the same substance. When the index of variation is positive or zero the median lethal dose

(L.D. 50) is always a positive real number, namely, the concentration of the substance, in suitable units, required to produce an expected 50% mortality; whatever the fluctuations of m it is possible to assign at least a probable value to the L.D. 50. But in many of the compounds studied the index of variation is negative, and this entails the possibility that the L.D. 50 may be negative, infinite, imaginary or complex. This is equivalent to saying that no concentration of the substance may suffice to produce a 50% mortality, but it follows that if the range of fluctuation of m is at all large the value assigned to the L.D. 50 may have no obvious meaning, though it remains a valid parameter fixing the characteristics

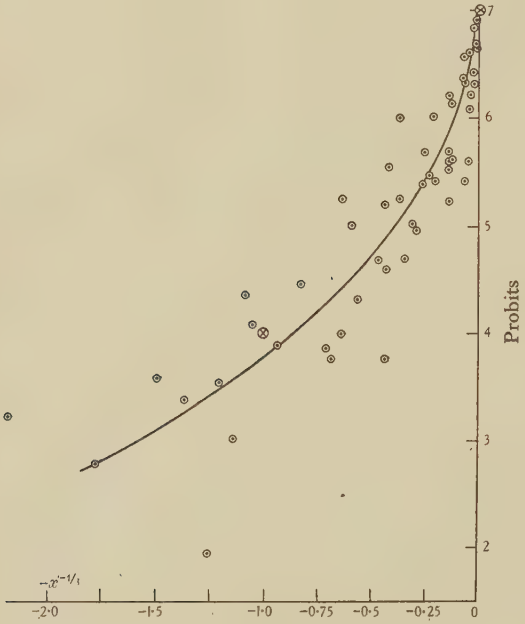

Fig. 1. Collation of results with methyl-derivatives using an incorrect value of α, viz.: $-\frac{4}{3}$.

of toxicity. Nevertheless, since the purpose of this work is to determine the relative value of the different compounds as fungicides, the L.D. 50, which can be directly interpreted in this sense, has been included in the tables rather than the less obvious modal tolerance. An imaginary value may be taken to imply absence of fungicidal potency.

Estimation of the index of variation

In only one case in the interpretation of the results was it necessary to compare different indices of variation, and since in this case the difference was 'obviously' significant no attempt was made to enter on the laborious computations described in the writer's previous work (1942*a*). On *a priori* grounds, however, it appears that the index of variation must always be a rational number, and is more likely to be a simple fraction than a complicated one.

To obtain the best estimate of the index, the results of separate experiments were combined by the following procedure: (i) the observed points were separated where necessary into their component curves (part of the activity of most of the substances was due to a component giving $\alpha=0$); (ii) for those belonging to the curve with $\alpha\neq 0$, x^α (x = concentration) was plotted against y (probit mortality) for an arbitrary α and the best straight line drawn by eye; (iii) the intercepts on $x^\alpha=0$ and $y=4$ were noted;

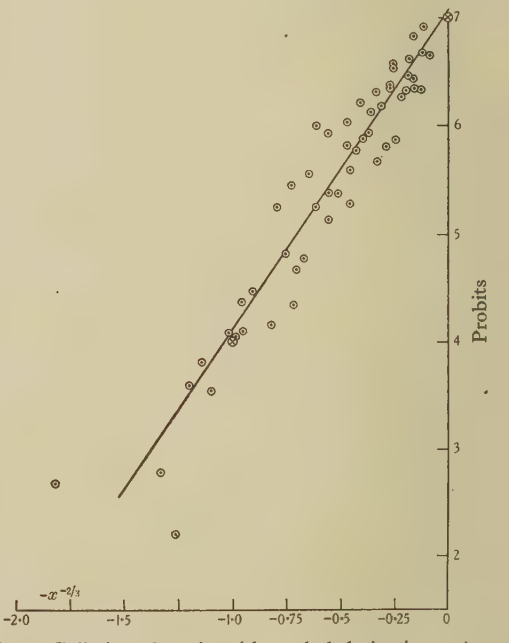

Fig. 2. Collation of results with methyl-derivatives using the value of α taken as correct, viz.: $-\frac{2}{3}$.

(iv) adjusted values of x^α and y were calculated so that the adjusted line should pass through

$$(x^\alpha=1,\ y=4) \text{ and } (x^\alpha=0,\ y=7).$$

The adjusted values for all the relevant experiments were then combined in one plot; incorrect values of α gave a marked curvature to this plot, as evidenced by Figs. 1 and 2 which were compiled from experiments on dimethyl derivatives. Estimations by this method were made on these results, and separately on all those obtained with other compounds. It was found that $\alpha=-\frac{2}{3}$ gave the best fit in both cases; $-\frac{1}{2}$ and -1 both gave curved plots.

The results obtained with tetramethylthiuram-disulphide did not agree with those from the other compounds; while closely fitting $\alpha=-\frac{1}{3}$ it cannot

be claimed that they are inconsistent with $\alpha=0$, but the former value has been assigned from theoretical considerations (see Fig. 3).

IV. PRESENTATION OF RESULTS

The results are presented in Tables 1–12 and a summary of the calculated statistics is given in Table 13. In Tables 1–9, x is the concentration applied in millimoles per litre of the compound named, q the proportional mortality of the spores thus treated, and N the number of spores counted. Where N is 500 in all treatments this figure is entered only once. In Tables 10–12, x is given in terms of 1% suspension. For the purpose of calculating the L.D. 50, as given in Table 13, it is assumed that the whole of the spray deposit under any one drop of spore suspension was in solution therein; this assumption is not strictly true. In addition to the compounds listed the following were tested but found insufficiently toxic to allow of their parameters being estimated: piperidinium chloride, dibutylammonium chloride, and dipentamethylenethiuram-disulphide.

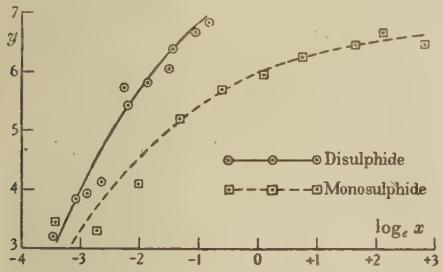

Fig. 3. Relation between probit mortality of spores exposed to thiuram-sulphides and logarithmic concentration, showing difference in curvature.

TABLE 1. *Sodium dimethyldithiocarbamate*

Exp.	x	q	N
5113	0·0109	0·000	200
	0·0218	0·054	150
	0·0436	0·139	250
	0·0874	0·445	250
	0·1748	0·649	500
	0·3497	0·880	500
	0·6994	0·931	500
5114	0·0409	0·003	500
	0·0612	0·201	
	0·0918	0·694	
	0·1382	0·839	
	0·2073	0·797	
	0·3105	0·767	
	0·4658	0·820	
	0·6987	0·936	
5115	0·0402	0·000	500
	0·0553	0·045	
	0·0713	0·114	
	0·0951	0·133	
	0·1268	0·312	
	0·1692	0·447	
	0·2256	0·629	
	0·3008	0·726	
	0·4011	0·822	
	0·5348	0·891	
	0·7131	0·839	
5116	0·394	0·446	500
	0·525	0·702	
	0·700	0·845	
	0·933	0·862	
	1·244	0·923	
	1·655	0·939	
	2·945	0·932	
	3·927	0·923	
	5·236	0·939	
	6·981	0·9825	

TABLE 2. *Sodium diethyldithiocarbamate*

Exp.	x	q	N
5132	1·831	0·036	500
	3·662	0·078	400
	7·324	0·170	500
	14·649	0·477	500
	29·299	0·795	500
5133	1·087	0·048	500
	1·630	0·097	
	2·445	0·160	
	3·668	0·195	
	5·502	0·550	
	8·253	0·855	

TABLE 3. *Sodium diisopropyldithiocarbamate*

Exp.	x	q	N
5232	2·208	0·000	500
	3·312	0·025	
	4·968	0·158	
	7·452	0·507	
	11·178	0·644	
	16·767	0·939	
	25·150	0·9959	

TABLE 4. *Sodium dibutyldithiocarbamate*

Exp.	x	q	N
5122	0·086	0·016	500
	0·172	0·047	500
	0·344	0·073	500
	0·688	0·170	500
	1·377	0·265	400
	2·754	0·414	400
	5·508	0·610	500
	11·016	0·804	500
	22·032	0·917	500
	44·064	1·000	500
5123	0·086	0·000	500
	0·172	0·001	
	0·344	0·018	
	0·688	0·082	
	1·377	0·102	
	2·754	0·161	
	5·508	0·357	
	11·016	0·537	
	22·032	0·880	
	44·064	1·000	

TABLE 4 (*cont.*)

Exp.	x	q	N
5124	2·484	0·265	500
	3·312	0·346	
	4·416	0·471	
	5·888	0·513	
	7·887	0·628	
	10·516	0·736	
	14·021	0·827	
	18·595	0·891	
	24·727	0·938	
	33·036	0·9960	
	44·048	0·9980	

TABLE 5. *Sodium pentamethylenedithiocarbamate*

Exp.	x	q	N
5152	1·707	0·102	200
	3·414	0·233	500
	6·828	0·443	500
	13·656	0·845	500
	27·312	0·9837	500
5153	0·474	0·145	500
	0·711	0·200	400
	1·068	0·233	400
	1·602	0·315	500
	2·403	0·316	450
	3·604	0·336	500
	5·406	0·552	500
	8·109	0·709	400
	12·162	0·831	400
	18·243	0·947	500
	27·365	0·9836	500

TABLE 6. *Sodium ethylphenyldithiocarbamate*

Exp.	x	q	N
5412	1·585	0·031	500
	2·377	0·036	
	3·565	0·078	
	5·348	0·078	
	8·022	0·177	
	12·033	0·275	
	18·050	0·391	
	27·070	0·593	
	40·604	0·663	
	60·906	0·742	
	91·359	0·861	

TABLE 7. *Dimethylammonium dimethyldithiocarbamate*

Exp.	x	q	N
5201	0·0013	0·004	500
counted	0·0025	0·018	
after 24 hr.	0·0051	0·011	
	0·0101	0·202	
	0·0202	0·436	
	0·0808	0·519	
	0·1618	0·670	
	0·3236	0·864	
	0·6472	0·908	
	1·2944	0·9748	
5202	0·0506	0·012	500
counted	0·0759	0·064	
after 48 hr.	0·1138	0·280	
	0·1706	0·338	
	0·2559	0·578	
	0·3838	0·634	
	0·5756	0·750	
	0·8631	0·885	
	1·2944	0·946	

TABLE 8. *Dimethylammonium chloride*

Exp.	x	q	N
5211	0·064	0·000	500
	0·096	0·066	
	0·144	0·222	
	0·216	0·228	
	0·324	0·265	
	0·485	0·347	
	0·727	0·426	
	1·090	0·533	
	1·636	0·475	
	2·453	0·456	
5212	0·485	0·000	500
	0·727	0·036	
	1·090	0·150	
	1·636	0·386	
	2·453	0·358	

TABLE 9. *Piperidinium pentamethylene-dithiocarbamate*

Exp.	x	q	N
5251	0·379	0·035	500
	0·568	0·058	
	0·852	0·160	
	1·278	0·238	
	1·917	0·253	
	2·876	0·336	
	4·314	0·582	
	6·471	0·746	
	9·735	0·794	
	14·802	0·904	
	21·903	0·9750	

TABLE 10. *Tetramethylthiuram-monosulphide*

Exp.	x*	q	N
5176	0·0010	0·065	600
	0·0020	0·045	
	0·0039	0·186	
	0·0078	0·583	
	0·0156	0·760	
	0·0312	0·837	
	0·0625	0·898	
	0·1250	0·927	
	0·2500	0·953	
	0·5000	0·931	

TABLE 11. *Tetramethylthiuram-disulphide*

Exp.	x*	q	N
5173	0·0156	0·142	600
	0·0312	0·622	
	0·0625	0·855	
	0·1250	0·9685	
	0·2500	1·0000	
5174	0·0088	0·036	600
	0·0132	0·130	
	0·0198	0·190	
	0·0297	0·746	
	0·0445	0·806	
	0·0667	0·918	
	0·1000	0·954	

TABLE 12. *Dipentamethylenethiuram-monosulphide*

Exp.	x*	q	N
5186	0·2694	0·015	600
	0·4041	0·098	
	0·6061	0·466	
	0·9091	0·627	

* In Tables 10–12 the unit of x = 1 % suspension.

TABLE 13. *Summary of results*

	Compound	α	U	$\frac{\chi^2}{n}M$	L.D. 50	n
1	$(CH_3)_2NCSSNa$*	$-\frac{2}{3}$	0·63	0·029	0·0108	30
2	$(C_2H_5)_2NCSSNa$*	$-\frac{2}{3}$	10·41	0·077	4930i	4
3	$(C_3H_7)_2NCSSNa$*	$-\frac{2}{3}$	0·41	0·109	8·00	2
4	$(C_4H_9)_2NCSSNa$*	$-\frac{2}{3}$	4·30	0·038	6·15i	5
5	$C_5H_{10}NCSSNa$*	$-\frac{2}{3}$	10·23	0·113	295i	6
7	$(CH_3)_2NCSS(CH_3)_2H_2$*	$-\frac{2}{3}$	0·33	0·159	0·0201	7
8	$(CH_3)_2NH_2.Cl$	$-\frac{2}{3}$	14·8	0·094	7·69	12
9	$C_5H_{10}NCSSNHC_5H_{10}$*	$-\frac{2}{3}$	438	‡	? 32·1	3
10	$(CH_3)_2NCS.S.CSN(CH_3)_2$	$-\frac{2}{3}$	0·88	0·009	0·197	7
11	$(CH_3)_2NCS.S_2.CSN(CH_3)_2$	$-\frac{1}{3}$	0·37	0·217	0·084	9
12	$C_5H_{10}NCS.S.CSNC_5H_{10}$	$-\frac{2}{3}$	0·33	0·183	1·60	1
1	$(CH_3)_2NCSSNa$	0	? 0·3	—	? 2·33	0
2	$(C_2H_5)_2NCSSNa$	0	—	—	29·2	3
3	$(C_3H_7)_2NCSSNa$	0	0·12	—	8·54	1
4	$(C_4H_9)_2NCSSNa$	0	1·23	—	6·6	20
5	$C_5H_{10}NCSSNa$	0	0·63	—	7·37	6
6	$C_2H_5C_6H_5NCSSNa$†	0	1·37	—	25·8	5
7	$(CH_3)_2NCSSN(CH_3)_2H$	0	? 1·5	—	? 0·25	7
9	$C_5H_{10}NCSSNHC_5H_{10}$	0	0·74	—	3·88	3

* These compounds behave as mixtures having two components differing in α.
† This compound gave indications of the $\alpha<0$ component inadequate to calculate its statistics.
‡ The formula $M=m^2/A$ is inaccurate when m is comparable with its standard deviation.

Significance of differences

$U_\alpha(x+y)$ denotes the variability estimated from the combined figures for compounds x and y:

$U_{-\frac{2}{3}}(7) \lessdot U_{-\frac{2}{3}}(3) \lessdot U_{-\frac{2}{3}}(1)$;
$U_{-\frac{2}{3}}(4) \lessdot U_{-\frac{2}{3}}(5) \lessdot U_{-\frac{2}{3}}(2) \lessdot U_{-\frac{2}{3}}(8) \lessdot U_{-\frac{2}{3}}(9)$.
$U_{-\frac{2}{3}}(1+7) < U_{-\frac{2}{3}}(4)$;
$U_{-\frac{2}{3}}(1+7) < U_{-\frac{2}{3}}(8)$;
$U_{-\frac{2}{3}}(1+7) \lessdot U_{-\frac{2}{3}}(10) \gtrdot U_{-\frac{2}{3}}(12)$;
$U_{-\frac{2}{3}}(12) < U_{-\frac{2}{3}}(5)$.
$\frac{4}{9}U_{-\frac{2}{3}}(1+7) > \frac{1}{9}U_{-\frac{2}{3}}(11)$.
$U_0(1) \lessdot U_0(5) \lessdot U_0(9) \lessdot U_0(6)$;
$U_0(3) < U_0(9)$; $U_0(5) < U_0(4)$.

All the above refer to $P=0{\cdot}05$.

V. DISCUSSION AND CONCLUSIONS

Fig. 4 shows the type of action found: these curves are plotted from the figures calculated back from the statistics given in Table 13. Only at the higher concentrations does the linear relationship between probit mortality and log-concentration hold. The figures in Table 13 for $\alpha=0$ apply to this part of the curves, while those for $\alpha<0$ apply to the lower part of the curves. Bliss (1939) showed that the effect of a mixture of poisons of constant relative concentration, each of which separately gives a straight line, should be represented on a graph of this type approximately by two intersecting straight lines; actually the curve in such a case is a hyperbola lying very close to the two lines as asymptotes. Since the data are here not sufficiently extensive to reveal the hyperbolic effect, the curves as given, showing a sharp break, may be taken as a sufficiently close approximation. The inference is that the dithiocarbamates act as mixtures of two components, of which one gives the curved portion ($\alpha<0$) and the other the straight line ($\alpha=0$). For this reason the two parts of the curves have been separated in Table 13 and separately calculated.

The following reactions take place when an alkali salt of a dithiocarbamic acid is exposed to hydrogen ions:

(i) $2NR_2CSSNa + 2H_2O \rightleftharpoons NR_2CSSNR_2H_2 + CS_2 + 2NaOH$,

(ii) $NR_2CSSNR_2H_2 \rightleftharpoons CS_2 + 2NR_2H$,

the alkali and amine liberated being neutralized by the acid present. Since the spore secretion has been shown* to have a slightly acid reaction, these reactions may be assumed to take place when the compound is exposed to a spore suspension; the two parts of the curves may then represent the action on the one hand of carbon disulphide and on the other of the dialkylamine or the undecomposed molecule.

The tendency of the dithiocarbamates to decompose in the above way increases in the order dimethyl- < diethyl- < dibutyl- < ethylphenyl-dithiocarbamate;† only vague indications of the linear phase of the toxicity were shown (at high concentration) by dimethyldithiocarbamate, whereas with ethylphenyldithiocarbamate no satisfactory figures could be obtained for the curved phase, the other compounds being intermediate (see Fig. 4). Hence,

* Unpublished work of Dr R. L. Wain and E. H. Wilkinson in this laboratory.
† Results communicated to the author by Dr M. E. D. Windridge.

it is probable that the linear phase represents the action of carbon disulphide, which was confirmed by an experiment using an emulsion of CS_2 in water, which, though suffering from grave technical defects owing to the instability of the emulsion, indicated a zero index of variation.

On the other hand, Tables 7, 8, 9, and 13 show that the dialkylammonium salts gave curves having the same index of variation as the dithiocarbamates in their lower phases. On these grounds the curved part of the graphs was referred to the action of the dialkylamino portion of the molecule.

If the critical range of concentration is low, the strength of the basic part of the molecule will hardly affect the *p*H at the spore surface; thus $W_0([H^+])$ will be independent of the base, and on either assumption $W_0(A)$ will depend solely on the extent to which the dithiocarbamate decomposes. It will, therefore, *decrease* with decreasing base strength. But if higher concentrations are required, a strong base combined with the CS_2 will tend to affect the *p*H at the spore surface, reducing $W_0([H^+])$. Judging by the range of K_b values in Table 14, this effect is likely to outweigh that of differences in the degree of

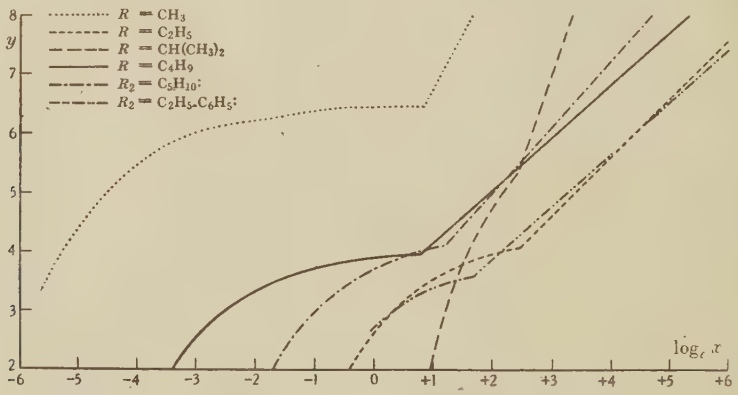

Fig. 4. Relation between probit mortality of spores in solutions of the compounds $R_2NCSSNa$ and logarithmic concentration in terms of millimoles per litre.

The mode of action of carbon disulphide

Since the variabilities of the spores to the component identified with carbon disulphide vary, it is clear that this substance reacts differently, according to the nature of the associated base. It is probable that the only reaction possible under the given conditions is hydration, which in dilute solution may be represented as:

$$CS_2 + H_2O \rightleftharpoons HOCSS^I + H^+.$$

The total quantity (A) of CS_2 present in a given volume being equal to the sum of the hydrated and unhydrated portions, we may write

$$A = [CS_2] + [HOCSS^I],$$

whereas the law of mass action gives

$$K[CS_2] = [HOCSS^I]\,[H^+]$$

$$\therefore A = [CS_2]\left(1 + \frac{K}{[H^+]}\right) = [HOCSS^I]\left(1 + \frac{[H^+]}{K}\right).$$

Applying the theory of variability we get, assuming $\frac{K}{[H^+]} \ll 1$,

either $W_0(A) = W_0([CS_2])$,

or $W_0(A) = W_0([HOCSS^I]) + W_0([H^+])$,

according as the substance is absorbed by the spores in the form of carbon disulphide or dithiocarbonate.

decomposition of the dithiocarbamates, so that, assuming that $HOCSS^I$ is the effective substance, $W_0(A)$ will *increase* with decreasing base strength, whereas if CS_2 is permeative, $W_0([H^+])$ is of no account, and the opposite tendency will prevail.

The observed order of $U_0(A)$ in Table 13 indicates that it increases with decreasing K_b, the one exception being with $(CH_3)_2NCSSN(CH_3)_2H_2$, in which the critical range is much lower (see L.D. 50). The observations, therefore, support the hypothesis that hydration of carbon disulphide is an essential step in its toxic action on *Macrosporium*. The structural analogy between $HOCSS^I$ and H_2NCSS^I may be noted.

The mode action of dialkylamines

An interpretation of the figures for the lower phase of the toxicity depends on the solvation equilibria of the amines; these equilibria are as follows:

$$\underset{(h)}{NR_2H + H_2O} \rightleftharpoons \underset{(b)}{NR_2H_2OH} \rightleftharpoons NR_2H_2^+ + OH^I,$$

the equilibrium constants being

$$K_h = \frac{[NR_2H_2OH]}{[NR_2H]\,[H_2O]}; \qquad K_b = \frac{[NR_2H_2^+]\,[OH^I]}{[NR_2H_2OH]}.$$

Most published figures for the dissociation of amines give the apparent dissociation constant K,

$= K_h K_b/(1 + K_h)$, whereas in order to use the theory of variability to understand the action of these compounds as fungicides it is necessary to know each constant separately, at least to an order of magnitude. For certain amines these figures have been given by Moore (1907) and Moore & Winmill (1912). Certain others can be deduced from the apparent K with the help of the considerations presented in those papers. Relevant figures have been assembled in Table 14.

It has already been indicated that for some dithiocarbamates the order of stability is the same as the order of K_b. Table 13 shows that the variabilities do not follow such an order; the following hypothesis explains, however, the observed order as far as the dithiocarbamates are concerned. The amines themselves are postulated to be non-permeative; the dithiocarbamates (or some related compounds) are permeative. The following sequence of equilibria is assumed:

$$NR_2H_2OH \rightleftharpoons NR_2H + H_2O$$
$$NR_2H + CS_2 \rightleftharpoons R_2NCSSH.$$

From this it would follow (Parker-Rhodes, 1942*a*, p. 131) that the variability will increase with the hydration of the amine, i.e. with K_h, and will also be greater the further the second equilibrium lies to the left. The first consideration leads to the order of variabilities $Bu_2- = iso\text{-}Pr_2- < Me_2- < C_5H_{10}- < Et_2-$, and the second to $iso\text{-}Pr_2- < Me_2- < C_5H_{10}- < Et_2- < Bu_2-$; whereas the observed order is $iso\text{-}Pr_2- < Me_2- < Bu_2- < C_5H_{10}- < Et_2-$. Clearly the effect of hydration preponderates for the lighter molecules, but for the dibutyldithiocarbamate the instability of the compound is sufficient to produce a significant increase in the variability. Since, however, there is no evidence of differences in the index of variation, there can be no major differences in the spontaneous occurrence of the second equilibrium but only in the extent which it is affected by the spore secretion.

The high variability observed with dimethylammonium chloride is explained by the inability of the spores to provide the necessary carbon disulphide; but that some other compound is formed which enables the amine to enter the spores is indicated by the fact that this salt is toxic at all. Nevertheless, the toxicity of the other amine hydrochlorides and sulphates is in general small, giving a low imaginary L.D. 50, and only in this one case could statistics be estimated.

The mode of action of thiuram-monosulphides

The variabilities of the spores to tetramethyl- and dipentamethylenethiuram-monosulphides are not significantly different from each other or from those to dimethyldithiocarbamates. Since the indices of variation are also the same, the compounds must act as though almost completely dissociated into two parts, each containing a single amino residue. This dissociation does not occur spontaneously and must, therefore, be due to the influence of the spores. This process would be expected to increase the variability over that observed with dimethyldithiocarbamates, if the latter is one of the products of fission. That it is not so indicates either that the increment in variability happens to be very small, or that the product of fission is some compound to which the variability is smaller than to dimethyldithiocarbamate.

In the case of dipentamethylenethiuram-monosulphide the variability is significantly *less* than that to the corresponding dithiocarbamate. In so far as the high variability to the latter can be attributed to the hydration of the free amine, so high a value would not be expected in the present case where, presumably, no free amine is produced. This result thus further demonstrates the importance of hydration in the action of amines.

The mode of action of thiuram-disulphides

The figures for tetramethylthiuram-disulphide show (*a*) that the index of variation is one-half of that for other compounds, and (*b*) that the variability of the spores is significantly less than four times that to dimethyldithiocarbamate or to tetramethylthiuram-monosulphide. It follows that the molecule is only slightly, if at all, dissociated in acting on the spores.

The results are in agreement with the hypothesis that the dithiocarbamates are absorbed only after oxidation to the thiuram-disulphides; on this view, however, the results with thiuram-monosulphides can only mean that the fission products of these

TABLE 14. *Solvation equilibria of the amines*

Amine	Formula	Moore & Winmill (1914) K_h	Moore & Winmill (1914) K_b	Moore & Winmill (1914) K	Hodgman (1935) K	Vogel (1939) K	I.C.T. (1929) K
Ammonia	NH_3	1·13	0·341	0·1805	0·180	0·180	—
Dimethylamine	$NH(CH_3)_2$	0·46	20·34	6·354	7·4	5·2	3·9
Diethylamine	$NH(C_2H_5)_2$	Large	11·36	11·360	12·6	—	10·1
Di-*n*-propylamine	$NH(C_3H_7)_2$	Very large	9·49	9·488	10·2	—	8·2
Di-*n*-butylamine	$NH(C_4H_9)_2$	Insoluble	—	—	—	—	—
Di-*iso*-butylamine	$NHCH(CH_3)C_2H_5$	Insoluble	—	—	—	—	3·9
Piperidine	HNC_5H_{10}	2·46*	16·87*	12·0*	16·0	13·0	12·5

All figures for K_b and K multiplied by 10^4; temp. 25° C.

* Moore (1907).

compounds are more readily combined to thiuram-disulphides than are the dithiocarbamates, and there does not seem to be any compound satisfying this condition. It is, therefore, more probable that the thiuram-disulphides are themselves reduced by the spores to some compound more readily absorbed than the dithiocarbamates. This compound is no doubt the same as that produced by fission of the monosulphides, the greater variability to the latter compound being due to its presumably assymmetrical mode of fission; the compound in question might be an ester. On this view the dithiocarbamates would require esterification before absorption. The complete series of reactions might then appear as:

$$R_2NH_2OH \xrightarrow{-H_2O} R_2NH \xrightarrow{+CS_2} R_2NCSSH \xrightarrow{+XOH} \xrightarrow{-X} R_2NCSSX \rightarrow \tfrac{1}{2}R_2NCSS_2SCNR_2,$$

where X represents some group supplied by the spores.

Dipentamethylenethiuram-disulphide proved insufficiently toxic to allow of its parameters of toxicity being estimated, owing to the difficulty of preparing concentrated suspensions.

Factors affecting the median lethal dose

An examination of Fig. 3 reveals a marked difference between the L.D. 50 for sodium dimethyldithiocarbamate and those for its higher homologues. Whereas the order of variabilities can be explained as dependent on the solvation equilibria of the amines, the values of this parameter appear (as is often the case) little related to the chemical properties of the toxicants. It seems that the superior toxicity of the dimethyl-derivative is related to the shortness of the hydrocarbon part of the molecule; which suggests that the monomethyldithiocarbamates might be even more toxic. A similar effect is observed among the thiuram-sulphides, and with the dialkylammonium salts.

In the case of the effect of the nitrogenous part of the molecule on the toxicity of the carbon disulphide, no definite conclusions can be drawn. It seems as though the presence of the ethyl group lowers the toxicity, but the effect is not very marked. The possibility of synergistic phenomena must not be overlooked.

Evaluation of dithiocarbamic acid derivatives as fungicides

As far as the fungus *Macrosporium* is concerned, only the methyl-derivatives show any promise of practical usefulness. Preliminary experiments with *Venturia inaequalis* (which have not been carried far enough for statistical analysis) suggest that this fungus is more sensitive, in that the pentamethylene-dithiocarbamate falls not far behind in the methyl-derivative laboratory tests.

Since the greater part of the toxicity appears due to the amino group, it should be pointed out that the negative index of variation is a severe limitation on the value of these compounds, since it implies that there is in any population a proportion of spores absolutely resistant to the toxic action of the amine. In sufficient concentration, of course, these spores will be killed by the carbon disulphide, and the question is raised whether this is the best means of utilizing the fungicidal properties of that compound; it is probably not so. Tetramethylthiuram-disulphide is relatively free from this objection since, though the index is negative, the proportion of spores absolutely resistant is only about 1 in 10,000.

Notwithstanding these objections, a high degree of partial toxicity may be valuable, and metallic salts of the dithiocarbamic acids may supplement this action with the toxicity of the metal itself. Work is in progress on such salts.

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Preliminary experiments on the use of oil-soluble copper compounds as fungicides

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Tests were made of the solubility of a number of copper compounds in mineral and vegetable oils. Phytocidal tests on onion foliage showed that turpentine, pine oil and white spirit were damaging whereas cottonseed oil and white oil were innocuous. Copper salts in white oil, white spirit, turpentine or pine oil showed a higher fungicidal value than in cottonseed oil. In laboratory tests, a solution of copper 3:5-di-*iso*propyl salicylate was more fungicidal than Bordeaux mixture of the same copper concentration. In a small field trial, copper 3:5-di-*iso*propyl salicylate dissolved in white oil to give a concentration of 0·01 % Cu was atomized three times at monthly intervals on to onion foliage without damage. The same material at 0·1 % Cu in white oil was phytocidal. No control of onion downy mildew (*Peronospora schleideniana*) was obtained in this trial. The method of atomization was convenient and practicable for small-scale operation. It gave perfect wetting of the foliage and was very economical of material.

INTRODUCTORY

The use of oils with copper sprays to act as stickers and penetrators is now a common practice. Fajans & Martin (1938) showed that the addition of cottonseed oil or of refined petroleum oil to sprays containing copper compounds in suspension increased both the initial retention and the tenacity of the spray deposit. The oil and the copper fungicide may be combined in a single spray by one of several methods. First, the oil may be prepared as an emulsion carrying the copper fungicide suspended in the aqueous phase, e.g. the copper oxychloride-white oil combination recommended by Orchard (1936). Or Bordeaux mixture may be prepared and a vegetable oil added (Martin, 1933), the Bordeaux precipitate acting as the emulsifier. By the addition of cuprous oxide to certain emulsions of white oils, oil-flocculated suspensions of copper oxide have been prepared (Fajans & Martin, 1938) in which the particles of the copper compound attach themselves to the oil globules in the emulsion.

The employment of an actual solution of a copper compound in oil is first recorded by de Ong (1932), who advocated the use of copper resinate $(C_{20}H_{29}O_2)_2Cu$ dissolved in a proprietary pine oil known as Palustrex. This solvent alone had certain fungicidal properties, and the copper resinate, in laboratory tests, was shown to be a slightly inferior fungicide to Bordeaux mixture. For use in the field, 16 gal. of Palustrex containing 25 % by weight of copper resinate was added to 80 gal. of a petroleum-oil emulsion. This mixture was diluted with 20 times its volume of water and tested against walnut blight and powdery mildew of cantaloupe melons. In the control of these diseases the copper-oil sprays were more effective than standard Bordeaux applications.

In 1935 de Ong claimed that the copper resinate-Palustrex mixture had been successfully used for 3 years in California, controlling fungus diseases at a lower copper concentration than was necessary in using Bordeaux mixture. Tests made on leaves and shoots of apricot and plum showed that approximately 20 % of the copper thus applied in oil was carried into the plant tissue by the penetration of the oil. No reference to phytocidal action is made in these two papers.

In this country, oil-soluble copper fungicides have been tested at Cheshunt, principally for use on rose foliage. Read (1934) reports that a 50 % solution of copper oleate in cottonseed oil could be emulsified at 60° C. with an equal volume of a 20 % glue solution. This emulsion diluted to 0·8 % with water effectively controlled rose mildew. Emulsified solutions of

copper oleate in white petroleum oil caused injury to rose foliage, thought to be due to penetration by the dissolved copper oleate. The same author in 1935 stated that slight injury to rose foliage was caused by the copper oleate-cottonseed oil spray emulsified and diluted as previously described. He notes that other oil-soluble compounds of copper, zinc and mercury had been tested, of which the oil-soluble mercury compounds were very potent fungicides. All these materials proved unsuitable for use because of their phytocidal action.

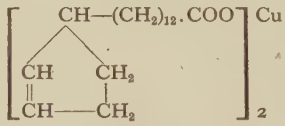
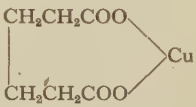
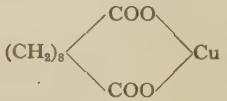
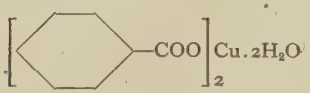
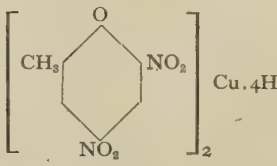
In the experiments noted above, the oils carrying the fungicides were emulsified, then, after dilution, were applied in the usual way as water-borne sprays. An alternative method of applying such oils is by atomization, a technique which has been widely applied in insect-pest control. This obviates the need for emulsification and addition of water and thus greatly reduces the volume of material required for a given application. The atomized oil readily wets waxy foliage on which a satisfactory cover cannot be obtained with water-borne sprays.

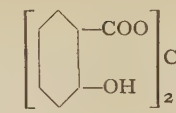
In the present investigation, a selection of oil-copper mixtures was examined for solubility and stability, and the more promising combinations were tested in the laboratory for fungicidal and phytocidal action. Particulars are also given of a small field trial carried out on onions with an atomized oil-soluble copper fungicide.

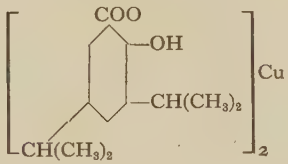
Materials

Copper compounds

The majority of the copper salts used were those of carboxylic acids of high molecular weight. This group comprised the oleate, palmitate, stearate, lineolate, chaulmoograte, sulphoricinoleate and abietate. Two salts of di-basic aliphatic acids were tested, the adipate and sebacate, and three in the aromatic group, the benzoate, salicylate and di-*iso*propyl salicylate. The remaining material employed was the di-nitro-*o*-cresylate. For the supply of these materials the authors are principally indebted to Dr R. L. Wain. The tentative formulae and copper contents as found by analysis are listed below:

Cu oleate	$(CH_3(CH_2)_7CH{=}CH.(CH_2)_7.COO)_2Cu$	9·77 % Cu
Cu palmitate	$(CH_3(CH_2)_{14}COO)_2Cu$	9·84 % Cu
Cu stearate	$(CH_3(CH_2)_{16}COO)_2Cu$	10·39 % Cu
Cu lineolate	$(CH_3(CH_2)_4CH{=}CH.CH_2CH{=}CH.(CH_2)_7COO)_2Cu$	8·9 % Cu
Cu chaulmoograte		9·82 % Cu
Cu sulphoricinoleate	$(CH_3(CH_2)_5.CH(O.SO_3H){-}CH_2CH{=}CH(CH_2)_7COO)_2Cu$	6·45 % Cu
Cu abietate		9·5 % Cu
Cu adipate		31·31 % Cu
Cu sebacate		23·87 % Cu
Cu benzoate		18·1 % Cu
Cu salt of dinitro-*o*-cresol		12·06 % Cu

Cu salicylate  20·39 % Cu

Cu 3:5-di-*iso*propyl salicylate  11·15 % Cu

The last-named was a sample of a commercial product, probably containing impurities.

Solvents

The characteristics of the oils used were as follows:

White oil. Shell no. P31 conforming to the specification of grade G (Martin, 1935); viscosity 141 sec. Redwood at 70° F.

White spirit. Glico no. 17602; sp.gr. 0·787; flash point, 100° F.; boiling range, 2 % at 161° C., 18 % at 170° C., 50 % at 180° C., 85 % at 190° C., 96 % at 200° C., final boiling-point 205° C.

Cottonseed oil. A grade of edible cottonseed oil of free fatty acid content below 1 %.

Turpentine. A commercial sample of Scandinavian origin.

'Palustrex' pine oil. The proprietary brand as used by de Ong (1932).

Solubilities

The materials under test were made up in the various oils at concentrations equivalent to 0·1 % Cu by weight. Samples were kept in glass flasks in the laboratory for at least 8 months to test stability and solubility. At the end of this time the majority of the materials were in the form of fine suspensions which had partially or completely settled out but were readily reformed on shaking. The exceptions were as follows:

Copper di-*iso*propyl salicylate in P31 which remained in true solution up to a concentration of at least 0·22 % Cu.

Copper chaulmoograte which could not be dissolved or suspended in any of the oils tested.

Copper palmitate and copper oleate in Palustrex which were reduced with the formation of metallic Cu on the sides of the flask.

Copper benzoate in turpentine which formed an encrustation at the base of the flask, unaffected by shaking.

The copper salt of dinitro-*o*-cresol which formed a coarse, rapidly settling suspension in both white oil and white spirit, even at the low concentration of 0·024 % Cu.

The suspensions formed by the remaining materials at strengths equivalent to 0·1 % Cu could be used for atomization provided that the material was kept agitated during the application. Copper di-*iso*propyl salicylate had the great advantage in physical properties that it could be used at the required strength in an atomizer not fitted with an agitator.

Phytocidal properties

Laboratory tests of phytocidal properties were made in March 1942 on leaves of White Lisbon onion seedlings 5 weeks old. The following materials were hand atomized on to the foliage, using groups of at least ten plants per treatment: cottonseed oil, turpentine, Palustrex, Glico white spirit, P31 white oil, P31 plus Cu D.I.P.S.* at 0·01 % Cu, water plus Cu D.I.P.S. at 0·01 % Cu.

The leaves sprayed with Palustrex collapsed within 2 min. of spraying: those treated with turpentine began to droop within 5 min. and were prostrate after 24 hr. After 48 hr. the white spirit-sprayed leaves were almost prostrate. Those sprayed with cottonseed oil, P31, Cu D.I.P.S. at 0·01 % Cu in water or in oil remained unaffected.

Field tests on a number of onion varieties are described later. These confirmed that P31 plus Cu D.I.P.S. at 0·01 % Cu caused no damage but showed that P31 plus Cu D.I.P.S. at 0·1 % Cu was phytocidal.

Fungicidal properties

In laboratory trials the fungi employed were conidia of *Macrosporium sarcinaeforme*, *Venturia pirina*, and *Botrytis* sp. from onion foliage. Tests were carried out with slides sprayed with the constant-pressure atomizer using the technique described in previous papers (Martin *et al.* 1942).

None of the solvents used alone exhibited fungicidal activities except Palustrex which, atomized on to the slide for 10 sec. at the standard rate of deposition, reduced germination of *Macrosporium sarcinaeforme* spores by approximately 50 %. Cottonseed oil, used with the oleate, abietate, sulphoricinoleate and D.I.P.S. reduced fungicidal properties by comparison with turpentine or white oil used with the same copper compounds.

In view of the non-phytocidal properties of the white oil and the superior solubility of Cu D.I.P.S., the majority of the laboratory tests was carried out

* Abbreviation used for copper 3:5-di-*iso*propyl salicylate.

with Cu D.I.P.S. in white oil, using strengths of 0·01, 0·003 and 0·001 % Cu in each test. As the laboratory testing of oil-soluble fungicides is a relatively novel procedure there is no oil-soluble copper compound accepted as a standard of comparison. The tests therefore included Cu D.I.P.S. in water and also Bordeaux mixture at equivalent copper strengths.

The materials were applied with a Mysto hand atomizer, each plant being well wetted from both north and south sides. The first treatment was on 29 May when the plants were 6 in. high. Approximately 25 c.c. of fluid was used for each set of three plots, the time required being 7–8 min. A second application was made on 26 June when the plants were 12–15 in. high, requiring 75 c.c. per treatment. At the third application on 31 July the plants were 18 in. high with 7–10 leaves per plant and required 150 c.c. per treatment. All applications were made in calm sunny weather but heavy storms closely followed the first.

TABLE 1. *Results of laboratory trials*

Test fungus	*Macrosporium sarcinaeforme*
Exp. no. 129	D.I.P.S. in oil = D.I.P.S. in water > Bordeaux mixture
,, ,, 204	D.I.P.S. in oil > D.I.P.S. in water = Bordeaux mixture
,, ,, 211	D.I.P.S. in oil > D.I.P.S. in water = Bordeaux mixture
Test fungus	*Botrytis* sp.
Exp. no. 325	D.I.P.S. in oil > Bordeaux
,, ,, 507	D.I.P.S. in oil > D.I.P.S. in water = Bordeaux mixture
Test fungus	*Venturia pirina*
Exp. no. 408	D.I.P.S. in oil > D.I.P.S. in water = Bordeaux mixture
,, ,, 513	D.I.P.S. in oil > D.I.P.S. in water = Bordeaux mixture

Comparisons of this type are somewhat unsatisfactory because the atomization of an oil on to a surface will obviously give different rates of deposition and spread from the application of water-borne suspensions. Furthermore, the physical relations between the spore-containing drop and the spray deposit will also be affected. Subject to these reservations, the above summary arranges these three preparations in order of fungicidal merit as shown by the series of laboratory trials of different dates. The sign > is used to signify 'more fungicidal than'.

Mildew attack

Mildew was first noted on 28 July. By 31 July, the date of the third application, it had spread widely. Counts of diseased plants made on that date gave the following results:

Plots	0	*X*	0·1	0·01	0·1	*X*	0	0·01	0·01	0	0·1	*X*
Diseased plants	4	13	34	15	9	13	17	39	36	27	24	30

TABLE 2. *Results of field trial*

Total for *X* plots (controls)	56
Total for 0 plots (P31 alone)	48
Total for 0·1 plots (P31 + Cu D.I.P.S. at 0·1 % Cu)	67
Total for 0·01 plots (P31 + Cu D.I.P.S. at 0·01 % Cu)	90

The fungicidal superiority of the oil-dissolved di-*iso*propyl salicylate over Bordeaux mixture of the same copper concentration was consistently shown throughout these laboratory trials.

Field trials

A field trial against onion downy mildew (*Peronospora schleideniana*) was carried out on a 56 × 12 ft. bed of Bedfordshire Champion onions in a private garden in Worcestershire. The materials used were:

(1) Shell P31 white oil.

(2) Shell P31 in which Cu D.I.P.S. had been dissolved to give a solution of 0·1 % Cu.

(3) Shell P31 in which Cu D.I.P.S. had been dissolved to give a solution of 0·01 % Cu.

Applications

The plot was divided into three blocks in each of which four strips were randomized, one for each of the treatments and one unsprayed. Each strip consisted of four rows each with twenty plants per row.

It is evident that the applications had no effect in checking the disease. There was a lapse of a month between the second application and the first appearance of the mildew, and in this period the effectiveness of the spray deposit may have been much reduced by weathering, while there was also considerable growth of new foliage which would not be protected at the time of attack.

Phytocidal effects

The white oil alone and the white oil plus 0·01 % Cu as D.I.P.S. had no phytocidal action, but the higher concentration (0·1 % Cu as D.I.P.S. in white oil) was markedly phytocidal. Two days after each spraying the foliage receiving this treatment showed cream-coloured pits followed by shrivelling of the leaves from the tips downwards. The affected plants rapidly recovered and a month after treatment were indistinguishable from those in the remaining plots.

The yields at the time of lifting are given on p. 183, where each plot number in brackets is followed first

by the number of bulbs and then by the total plot yield:

X plots	(1) 56, 12 lb.;	(2) 72, 26 lb.;	(3) 45, 23 lb.
0 plots:	(1) 58, 15 lb.;	(2) 64, 17 lb.;	(3) 67, 16 lb.
0·1 plots	(1) 60, 13 lb.;	(2) 74, 15 lb.;	(3) 74, 18 lb.
0·01 plots:	(1) 62, 13 lb.;	(2) 63, 16 lb.;	(3) 80, 21 lb.

An analysis of variance of these plot yields results as follows:

	S.S.	Degrees of freedom	Variance
Total	201	11	18·3
Treatments	45	3	15
Blocks	90	2	45
Error	66	6	11

showing that there was no significant difference in yield due to treatments.

A subsidiary trial of phytocidal action was carried out on a neighbouring bed of onions containing varieties Ailsa Craig, Southport Yellow Globe, Danvers Yellow Globe, Bedfordshire Champion, Giant Zittau. A strip running transversely across these varieties was atomized with Shell P31 and another parallel strip with Shell P31 plus D.I.P.S. at 0·1 % Cu. The treatments were carried out in the same way and at the same dates as in the main trial. The damage recorded in this trial was similar to that observed in the other plot: no differences in varietal susceptibility to phytocidal effect were shown.

Discussion

The accepted method of combining a copper fungicide with an oil-containing spray has been by the addition of a water-borne suspension to an oil emulsion. The suspension remains in the aqueous phase until the latter evaporates on the sprayed surface, leaving particles of the fungicide attached to the oil film. A more intimate contact between oil and fungicide would obviously be secured if the copper compound were actually dissolved in the oil thus utilizing to the full the penetrating and sticking properties of the oil carrier. The investigations of Martin *et al.* (1942) have so widened the range of potential copper fungicides that a number of oil-soluble materials are now available. The present investigation shows that a di-*iso*propyl salicylate is an example which gives stable oil solutions of high toxicity, but there is no reason to suppose that the compound is unique in this respect. With further investigations it is probable that the existence of a range of such oil-soluble copper toxicants will be disclosed.

The use of oils on foliage is restricted mainly by considerations of phytocidal action. Grade G mineral oils, and vegetable glyceride oils such as cottonseed oil, have been used for some years on the foliage of fruit trees, hops and potatoes. The present paper shows that these oils may also be used on onion foliage but that white spirit, turpentine and pine oil are damaging. The effect of dissolved copper on the phytocidal action of oils has been tested only for the single case of Cu D.I.P.S. in white oil on onion foliage. Here it was shown that the solution was safe up to a copper concentration of at least 0·01 %, but unsafe at 0·1 % Cu.

The difficulty of obtaining a spray cover on the waxy foliage of the onion is well known, and the usual methods of combining oils with copper suspensions do not give satisfactory results. Onion plants were therefore used in a preliminary trial to explore the possibilities of atomizing undiluted oil containing copper in solution. The method was shown to give perfect wetting of the foliage and to be simple and expeditious for small-scale work: it was most economical in material. The disease against which the test was made was not ideal for an experiment of this type, since onion mildew is partly systemic, and the actual extent of air-borne infection from plant to plant in the trial plot was not known. Usually, however, infection with this disease spreads from a few plants initially attacked, and in the trial described in this paper it appears likely that this spread took place just before the final application of the fungicide. In future trials the attempt will be made to maintain a more effective protection by more frequent applications at the critical period.

The field trial was carried out by kind permission of Mrs Gurney, whose gardener, Mr Astley, gave valuable assistance. The authors also wish to express their gratitude to Dr H. Martin and Dr R. L. Wain who readily provided materials and information. The original sample of copper 3:5-di-*iso*propyl salicylate was supplied by Technical Products Ltd.

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Experiments on the rate of spread of narcissus stripe in the field

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Striped and healthy bulbs of several varieties of narcissus were interplanted: the amount of spread of the disease was greatest when the bulbs were planted close together. The results obtained are in accordance with McWhorter's view that transmission takes place through injured roots.

The disease (or group of diseases) known in England as stripe of narcissus and in America as narcissus mosaic or gray disease is established among commercial stocks. Successful sap inoculation by various methods (see Moore, 1939) has shown that the disease is due to a virus or virus complex (Caldwell & James, 1938) but the natural mode of transmission in the field is unknown. In England, Hodson (1932, 1935) suggested that thrips might be the vector but his experimental results were negative. Gould (1935) showed that the disease is not spread during hot-water treatment. In America, McWhorter & Weiss (1932) reported a 25% spread in one year where diseased and healthy bulbs were interplanted. McWhorter (1932) suggested that transmission was most likely to be due to contact between the roots of diseased and healthy bulbs in the ground or to an undiscovered insect vector. In support of the former view he cited an instance where, in roguing out diseased bulbs, the roots of 62 healthy plants were exposed and injured, with the result that 16 showed disease symptoms in the following year. He recommended that when diseased bulbs are rogued the two adjacent bulbs should also be removed. Haasis (1939), who concluded that very little transmission was due to flower picking, root damage during roguing, or contact between roots of diseased and healthy plants, suggested that spread of the disease was most likely caused by insects, and Blanton & Haasis (1939) reported transmission of the virus by aphides in the U.S.A. Caldwell & Prentice (1943) supported this view since in one experiment spraying with nicotine reduced the number of new infections visible the following season.

A preliminary experiment, laid down in 1931 by Dr P. H. Gregory at the Imperial College of Science, Biological Field Station, Slough, in which rows of striped King Alfred were alternated with rows of healthy Emperor, showed no spread of the disease in 5 years. More experiments were laid down by the writer in 1933. Later it became known that the disease was being studied elsewhere and, with one exception, no more experiments were undertaken at Slough.

Striped bulbs were planted in rows alternating with rows of sound bulbs of the same or, occasionally, of a different variety. The distance between the rows was 12, 9 or 6 in. and that between adjacent bulbs in a row 6 in. or occasionally 4 in., other healthy bulbs being planted at a distance from the diseased lots as controls. Charts were made each year showing the positions of striped plants. Flowers from striped and healthy plants were picked on different days. The experiments in which the bulbs were planted closest together (4 in. apart) were discontinued after 1938 as it became impossible to distinguish individual plants. The results are given in Table 1.

Table 1

Var. (sound bulbs)	Distance between bulbs in row (in.)	Distance between rows (in.)	No. from sound stock	No. from striped stock	No. striped in 1934	Increase in no. of striped plants			
						1934–8	1939	1940	1941
Henry Irving	6	12	10	5	3	0	0	2S, 1N	0
Emperor	6	12	14	7	4	0	0	0	0
Barrii conspicuus	6	12	20	10	5	0	2S	0	0
Spring Glory	6	12	20	10	9	0	1S	0	0
Grandis	6	12	40	40*	22	0	1S	0	1S
Emperor	6	12	60	60*	8	3S, 2N	—	—	—
Double Incomparabilis	4	9	90	50	49	2S, 2N	—	—	—
Leedsii Evangeline	4	6	36	24	9	6S, 7N	—	—	—

* Var. Victoria.

S = transmitted to plant in same row. N = transmitted to plant in next row.

The six lots in which the bulbs were farthest apart, five showed no spread of the disease by the fifth growing season after planting. Four of these showed a slight increase in the number of diseased plants during the next 3 years. The newly infected plants, with one exception, were adjacent to originally diseased ones. This spread may have been due to the entanglement of roots of healthy and diseased plants. Insect transmission is also a possibility but is less likely in view of the absence of spread during the first 5 years. The sixth experiment

with this spacing was moved to a fresh plot at the end of the first growing season. The following year there was an increase in the number of diseased plants suggesting that, as in McWhorter's experiment, transmission occurred through damage to the roots in lifting. No further spread had occurred by 1938 when the experiment was discontinued since the vigorous growth of the Emperor plants made it difficult to distinguish individuals. In the two remaining experiments where the bulbs were planted closer together some spread of the disease (both within the rows and from row to row) occurred during the first 5 years, this being most marked in the experiment in which the closest spacing was used. The control bulbs planted at a distance remained free from stripe.

In 1938 a few bulbs of a white triandrus hybrid (referred to as W.T.H.), all of which showed a severe stripe, were interplanted with sound stocks of Seagull and of a double Incomparabilis variety as follows:

Rows 1 and 2. 10 W.T.H. and 10 Incomparabilis in pairs, touching.

Rows 3 and 4. 10 W.T.H. and 10 Seagull in pairs, touching.

Row 5. 20 Incomparabilis in pairs, touching.

Row 6. 20 Seagull in pairs, touching.

Row 7. 10 W.T.H. planted singly.

In addition 20 bulbs of each of the sound stocks were planted at a distance as controls. The distance between adjacent rows was 9 in.

TABLE 2

Var. and position (each lot 20 bulbs)	No. of striped plants			
	1939	1940	1941	1942
Seagull, control	0	0	0	0
Seagull (row 6)	0	0	0	0
Seagull (rows 3 and 4)	1	1	2	2
Incomparabilis, control	0	0	0	0
Incomparabilis (row 5)	0	2	2	3
Incomparabilis (rows 1 and 2)	1	5	8	9

The results (Table 2) show that the controls planted at a distance remained sound. A few bulbs of the Incomparabilis variety planted in a row adjacent to a row of striped plants developed the disease. The sound bulbs in contact with diseased ones showed only two infected plants of var. Seagull but nine of the Incomparabilis variety which may be more susceptible.

It is not yet clear whether, under English conditions, the virus is spread by insects or by other agencies. Blanton & Haasis (1939) showed that the disease was spread by four species of aphides in the U.S.A. Caldwell & Prentice (1943) obtained more diseased plants in a plot surrounded by a wooden frame, to prevent intermingling of roots with those of adjacent diseased plants, than in a similar plot not so protected but sprayed with nicotine. No disease symptoms were seen in 1936–7–8 but these appeared in 1938–9 (the year following spraying) in 14% of the unsprayed and 3% of the sprayed plants. It was not stated whether the year 1937–8, in which infection presumably occurred, was a peak year for aphis infestation of narcissi in south-west England and no account was given of the occurrence of any insects on the foliage. In an experiment by the writer involving the use of a similar wooden frame for a different purpose the wood was rotten after 3 years and was harbouring a large population of slugs, woodlice, etc. which might conceivably spread the disease through injured roots.

The results of the present paper concur with McWhorter's view that root transmission takes place when diseased and healthy plants are inter-planted. Thus, the amount of spread is correlated with the distance between diseased and healthy bulbs, spread increased in old plantings where roots may have been more closely intermingled, and an increase in the number of striped plants followed the lifting and replanting of a mixed lot of diseased and healthy plants. No insects were observed attacking the foliage and, while the presence of an insect vector is not excluded, the small amount of infection of plants only 9 in. from diseased bulbs does not suggest transmission by insects above ground. The results of Caldwell & Prentice (1943) show a more rapid spread than that described in the present paper, suggesting that insect transmission may take place more freely in Devonshire than in the drier climate of Slough. The possibility remains that transmission may take place through roots or bulbs damaged by slugs, soil-inhabiting insects, or nematodes, particularly where roots of diseased and healthy plants are closely interwoven.

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On the control of potato skin spot disease

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The disease of the potato tuber, caused by *Oospora pustulans* Owen & Wakef., is of some importance for two reasons. First, certain varieties, such as King Edward, Majestic, Kerr's Pink, Arran Banner and Sharpe's Express, may be infected to such a degree that ware tubers are so disfigured as to affect their potential sale and, secondly, King Edward and Majestic are specially liable to 'blindness' due to invasion of the eyes by the skin spot fungus. The degree of 'blindness' varies considerably but on numerous occasions serious blanking after planting has been associated with this disease. Investigations by another worker carried out in Scotland between 1931 and 1934 had indicated little promise of controlling this disease by treatment of tubers at lifting time. Greeves & Muskett (1939), however, obtained excellent control of this disease by dipping tubers in solutions of organo-mercurial compounds for a period of ½–1 min. Dipping had to be done at lifting time for the best results or, at the latest, before the elapse of a period of 8 weeks from lifting for any substantial control.

Similar results were obtained (Foister, 1940) over a period of years for the control of dry rot of potato, due to *Fusarium caeruleum*, and therefore it was decided to test the effectiveness under Scottish conditions of these organo-mercurial compounds against skin spot on varieties different from those used by Greeves & Muskett. The experiments are briefly outlined and the results are tabulated without much discussion, since they amply prove that Greeves' & Muskett's recommendations apply equally to Scottish conditions and to varieties other than those used by them.

Four cwt. King Edward tubers, ware and seed, were dipped on 22 Oct. 1941, for 1 min. in an organo-mercurial solution, 'A', and 4 cwt. in another organo-mercurial solution, 'B'. When dry, these were pitted next to a large quantity of many tons of undipped tubers. Five cwt. Kerr's Pink tubers, ware and seed, were similarly dipped on 18 Oct. 1941, in solution A, 5 cwt. in solution B and 5 cwt. left untreated: all were pitted in one clamp. These two varieties were left undisturbed until examined for skin spot development on 31 Mar. 1942 and 8 Apr. 1942 respectively. Samples were taken as representative of the total as possible (Table 1).

One cwt. of each of the three sets of King Edward tubers was planted on 22 Apr. 1943, at East Craigs in a field known to be relatively free from skin spot, receiving the normal potato cultivation and manuring. Table 2 shows the rates and differences of emergence above soil level of the three sets, demonstrating that dipped tubers had a quicker and stronger emergence than undipped tubers and that the final percentage emergence of the dipped sets showed an advantage of 11·4 to 12·2%.

An approximate estimate by the farmer of blanking in the field where the main commercial stock was planted was: dipped stocks, 0·5–1%; control, 10%.

Table 1. *Degrees of skin-spot development*

Variety	Treatment	% free	% slightly affected	% moderately affected	% severely affected	Total no. of tubers	Total no. of tubers examined
King Edward	Control	2·43	60·52*	36·40*	0·63	Large tonnage	945
	Organo-mercurial A	87·65	12·34†	—	—	2000 approx.	405
	Organo-mercurial B	90·60	9·40†	—	—	2000 ,,	500
Kerr's Pink	Control	11·86	77·71		10·43	2500 ,,	700
	Organo-mercurial A	98·51	1·49	—	—	2500 ,,	1007
	Organo-mercurial B	97·90	2·10	—	—	2500 ,,	1300

* Mainly in the eyes, many of which were 'apparently' dead. † Infection was very slight.

Table 2. *Rates and percentage emergence of dipped and undipped King Edward tubers*

Treatment	6 June	16 June	28 June
Control	64·6	77·6	87·8
Organo-mercurial A	92·6	97·3	99·2
Organo-mercurial B	94·4	98·0	100·0

The weights of the resulting crops as shown in Table 3 is a still better reflexion of the advantage of dipping.

The seed sizes 1¾ in. and 1¼ in. collectively yield: organo-mercurial A, 100·00 lb.; organo-mercurial B, 110·15 lb. and control, 67·53 lb., or a loss of 32·47–42·62% of potential seed. It is interesting to note that the total weight has an apparent relation-

ship with the percentage emergence, e.g. loss of emergence, 11·4–12·2%; loss of crop, 11·49–11·8%.

These figures might not be repeated exactly in other seasons under different conditions but it is believed they are reasonably indicative of what might be expected in seasons and soils conducive to skin spot development.

The assistance of Mr J. Mitchell, Mr A. Pratt and Mr R. J. Scott is gratefully acknowledged.

TABLE 3. *Total weights (lb.) and percentage weights (organo-mercurial A = 100) of King Edward tubers at East Craigs*

	Over 2 in.		1¾ in.		1¼ in.		Chats		Total	
Treatment	Total weight	%	Total weight	%	Total weight	%	Total weight	%	Total weight	%
Organo-mercurial A	730	100·00	279	100·00	180	100·00	25¾	100·00	1214¾	100·00
Organo-mercurial B	678	92·87	300	107·52	203	112·77	29	112·62	1210	99·69
Control	744	101·91	196¼	70·34	116½	64·72	15¾	61·71	1072½	88·20

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Note: Virus nomenclature

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During recent years a number of specialists have expressed their views on the classification and nomenclature of plant viruses (see Valleau, *Chron. Bot.* (1941), **6**, 223; (1942), **7**, 152), and the object of this note is to draw attention to certain more general aspects of the problem which, in the opinion of the writer, merit consideration.

It is probably true to say that the average plant pathologist has as little interest in the classification of viruses as in that of fungi. In the course of his daily work he deals with a relatively few pathogens for which, in order to relate his work to those of others, he has to use scientific names. He desires these names to be (1) internationally acceptable, and (2) reasonably stable. At the present time virus names fulfil neither of these requirements, and the creation of names which do is dependent on the co-operation of virus workers and on international agreements which will, it is hoped, be world-wide. A pre-requisite for any system of virus nomenclature is a set of rules, analogous to the International Rules of Botanical and Zoological Nomenclature, governing the type of names to be used and the method for their valid publication. The particular form of name finally chosen, whether Latin binomials, which are finding favour at the moment, or some other, is of secondary importance. It is, however, essential that these rules cover viruses of both plants and animals; and they might usefully place an obligation on an author naming a virus to register (and so validate) the name and place of publication with a central authority, or a national branch of such an organization, which would at regular intervals publish lists of new names. But rules of nomenclature, unfortunately, are no guarantee of stability. They merely determine the validity of names and ensure that a species has but one valid name in each genus in which it may be placed. Also, rules give no guidance as to which valid name should be selected, and there is no reason to suppose that individual viruses will be given fewer valid names than fungi and bacteria. It is probable that the plant pathologist of the future will as rarely feel competent to make a decision on a point of taxonomy or nomenclature of viruses as the pathologist of to-day does among fungi. He will either follow established custom or rely on the expert systematist of his choice.

To a systematist the distinction between a pathogen and a non-pathogen is frequently irrelevant, and the result of his studies may involve changes in the names of important pathogens. It is, however, not always incumbent on pathologists to accept such changes. In 1939 over forty Societies, Offices, and Institutes in the British Isles agreed to use in their official publications the names of the fungi given in the *Common Names of British Plant Diseases* compiled by the Plant Pathology Committee of the British Mycological Society (see *Trans. Brit. mycol.*

Soc. **23**, 271, 1939) on the understanding that the names were in accordance with the International Rules and that they were kept under continual review. By such action a common usage was assured, and the individual pathologist was absolved from the responsibility of considering the adjustment of the names of the commoner pathogenic fungi before every change in the systematic breezes, while changes which in the opinion of the Committee reflect advances in mycological knowledge are incorporated at the earliest possible moment. This type of national agreement was a new development and one which might very well be considered for adoption both by other countries and internationally. The selection of standard scientific names for viruses is work for the future, but at the present time there is need for a more stable framework for the documentation of the virus literature than that provided by any individual's system of nomenclature. This requirement could in a large measure be met, throughout the English-speaking world at least, by the standardization of the common names for the diseases, from which common names for the viruses can always be derived. Many common names are already very widely accepted and prove the surest way to trace a particular virus through the mazes of the literature; and most of them will remain in everyday use after scientific names for the viruses have been agreed upon. Such a standardization would greatly facilitate the work of official plant pathologists who could then view with equanimity the necessary nomenclatural experimentation of virologists.

The multiplicity of virus names is constantly raising questions of synonymy to which E. W. Mason's clarifying concept of 'obligate' and 'facultative' synonyms (*Trans. Brit. mycol. Soc.* **25**, 433, 1942) frequently finds application. Synonyms are alternative names for a taxonomic group. Obligate synonyms are names based on the same type, that is to say they are synonyms by definition; facultative synonyms are names based on two or more types which are, in the opinion of one or more authorities, but not necessarily all, synonymous. In brief, facultative synonyms are matters of opinion, obligate synonyms are not. Although the type method is still impracticable for most viruses, these two kinds of synonyms are nevertheless easily recognized. For example, in the following partial list of the names of the tomato spotted-wilt virus

Two groups of facultative synonyms

Obligate synonyms:
- Tomato spotted-wilt virus Samuel *et al.* 1930
- 'T.S.W.' virus Ainsworth *et al.* 1934
- *Lycopersicum* virus 3 K. M. Smith 1937
- Tomato virus 1 J. Johnson in Smith 1937
- *Lethum australiense* var. *typicum* Holmes 1939 (tomato spotted-wilt virus, typical strain)
- *Lethum australiense* Holmes in Valleau 1940

Obligate synonyms:
- Pineapple side rot (virus) Sideris 1927
- Pineapple yellow-spot virus Illingworth 1931
- *Ananas* virus 1 K. M. Smith 1937

the first six names are obligate synonyms of one another for all the authors intended to name the same virus. For a like reason the three names for the pineapple virus are obligate synonyms of one another. In 1940 Sakimuri concluded that the pineapple yellow-spot and tomato spotted-wilt viruses are identical, so that in his opinion names in the first group are synonymous with those in the second, but this synonymy is only facultative.

It is urged that this distinction should be made when compiling lists of synonyms in order that *certainty* may not be confused with what may be only a compiler's *opinion*. This can best be done by citing the authority for the synonymy of a facultative synonym, e.g. if the pineapple yellow-spot virus is given as a synonym of tomato spotted-wilt the facultative nature of this synonym should be indicated by the addition of '*fide* Sakimuri'.

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Proceedings of the Association of Applied Biologists

Ordinary meeting of the Association of Applied Biologists held at 11.30 a.m. on Friday, 4 December 1942, at the London School of Hygiene and Tropical Medicine; the President, Dr Hubert Martin, in the Chair.

Symposium on the honey-bee

The following papers were read:

The position of the honey-bee in the national economy. Adult bee diseases. By C. G. Butler, M.A., Ph.D.
Brood diseases of the honey-bee. By P. S. Milne, B.Sc.
Orchard spray poisoning of the honey-bee. By G. A. Carter.
Work on repellents. Management of colonies for pollination. By C. G. Butler, M.A., Ph.D.
The design and interpretation of bee experiments. By D. J. Finney, M.A.
A bee counter for use in studies of bee activity. By A. C. Fabergé.

The position of the honey-bee in the national economy. Adult bee diseases

By C. G. Butler, *Rothamsted Experimental Station, Harpenden, Herts*

The position of the honey-bee in the national economy

It is commonly accepted that the value of the honey-bee lies in the honey it produces, and that bees are of more value to their owners than to the nation. However, while it is perfectly true that honey is the main product of beekeeping from the beekeeper's point of view, it can be shown that it is, from the national standpoint, a by-product only. The main function of beekeeping in the national economy, both in peace and in wartime, is the provision of pollinators for fruit and seed crops. If this were not so there would be little justification for the wartime allocation of an average of 15 lb. of sugar per annum to each colony of bees in this country. It is justified by the enormously greater value of the fruit crops, which, were it not for the bees, would in many seasons very largely fail.

The allocation of sugar for bees in England and Wales apparently amounts to an annual average of approximately 3000 tons, based upon a recent estimate involving nearly 55,600 beekeepers controlling about 429,000 colonies of bees. Many beekeepers maintain that bees produce a greater amount of sugar, in the form of honey, than they are fed, and that this 'honey-sugar' is more nutritious and beneficial than commercial sucrose or glucose. In fact, an annual average of 40–50 lb. of honey per colony may reasonably be expected at the present level of management, if legitimate surplus only is removed. Each colony will require, apart from the 20–25 lb. of honey which should be left in the hive, an additional 15–20 lb. of cane sugar or its equivalent for its maintenance during the non-productive months of the year. Each pound of honey contains approximately 75 % of invert sugar, the remainder being mostly water. Thus for 20 lb. of sugar fed to the bees per annum we can expect to obtain 30 lb. of sugar in the form of 'honey-sugar'. Particularly under wartime conditions, the production of 30–35 lb. of 'honey-sugar' in this country for the expenditure of 20 lb. of granulated sugar fed to the bees, is well worth while. This honey, contrary to popular belief, contains no measurable quantity of vitamins or other valuable accessory food substances, with the possible exception of iron and one or two other salts. It is no more than a palatable solution or crystalline suspension of invert sugars. Its palatability and natural invert sugar make it readily assimilable and of particular value to invalids, convalescents and children.

Honey production, however, has been overemphasized at the expense of the much more fundamental aspect of the bees' work, pollination. During 1941, when there was scarcely any yield of honey in most districts, the sugar allocation was not wasted, as the bees' pollinating activities were of very great

value. Small-scale studies of the numbers and kinds of pollinating insects present in certain orchards in Hertfordshire and Bedfordshire when the trees were in flower, showed that the only pollinating insects present in any numbers during this abnormally cold spring were honey-bees. Many incipient colonies of bumble-bees had been killed, and those that had survived were very backward. Solitary bees also were almost absent.

There is good evidence that in many places where large orchards or large areas of insect-pollinated seed crops are planted, there are insufficient wild pollinating insects present for full production. Apart from this crowding effect of crops which upsets the ratio between pollinators and flowers requiring pollination, the methods employed in intensive cultivation, such as ploughing up of grassland, and undercropping and clearing of hedgerows, destroy the nests and habitats of many wild pollinating insects, and their numbers are likely to have been greatly reduced since the beginning of the war. Large numbers must also be destroyed annually by insecticidal sprays. More data on this subject, which can only be obtained by means of large-scale surveys in the areas most concerned, are very desirable. Increased yields of as much as 50–100% have been obtained by growers making proper use of bees as pollinators, but it is greatly to be regretted that many British growers have not yet realized their value in this respect. In Europe generally, as well as in Russia, America and New Zealand, it is well recognized that the honey-bee is a valuable pollinator of both hard and soft fruits, and of important seed crops such as clovers, sainfoin, lucerne, mustard and buckwheat. Fortunately the position in this country is gradually changing, and the value of the honey-bee as a pollinator is slowly being realized both by horticultural experts and growers.

In some cases, introduction of honey-bees into orchards for purposes of pollination seems to have resulted in no obvious improvement in crop. This is almost certainly due to lack of understanding of the problems involved, and use of an insufficient force of bees. Very frequently, too, there has been the factor of competition between the trees and weeds, such as dandelions, growing in or near the orchards.

It is primarily the small backyard beekeepers, scattered throughout the country, who play such an important part in providing the necessary pollinating insects. However, a study of the distribution of colonies of bees in relation to agricultural land in England and Wales shows that only in three counties, Hertfordshire, Middlesex and Surrey, are there more than three colonies of bees per 100 acres. The north and west are particularly short of bees, many Welsh counties having fewer than 0·6 colony per 100 acres of agricultural land. There is no evidence of any concentration of colonies of bees in the predominantly fruit-growing areas.

The honey-bee is the only pollinating insect whose numbers can readily be controlled, and which can be taken in large numbers to those places where its activities are most desirable. There is little doubt that the honey-bee is the most important insect pollinator, and that its presence is indispensable if good fruit crops are to be obtained, particularly in cold seasons when the wild bees are backward, and in areas where large acreages of fruit and insect-pollinated crops are being grown. A recent estimate has shown that the annual average cash value of the honey-bee to the nation, assuming that it is responsible for pollinating about half the commercial fruit crop (a lower estimate than that usually employed) is at least £5,287,000. Of this £4,000,000 is the wholesale value of the fruit pollinated and £1,287,000 the value of the honey produced. From these figures it can be calculated that the annual value of each colony of bees to the nation is about £12.

Adult bee diseases

During recent years large numbers of samples of adult bees have been received at Rothamsted Experimental Station for examination. About one-quarter of these samples were suffering from acarine disease, a complaint caused by infestation of the thoracic tracheae by the mite, *Acarapis woodi*. It would, however, be unfair to deduce from these records that 25% of all colonies of bees in England and Wales are suffering from acarine disease, as samples voluntarily sent for examination may be expected to be biased from several causes.

In the autumn of 1941 it was decided, with the concurrence of the Ministry of Agriculture and Fisheries, that an attempt should be made, by means of a survey, to obtain an estimate free from bias of the incidence of adult-bee diseases. In the absence of any complete register of beekeepers it was impracticable to attempt to include all those in England and Wales. The survey which was eventually carried out was based on members of Beekeepers' Associations only, probably about one-third of the total number. This may have resulted in a bias towards the keener and better beekeepers, but the effect is probably not very great. The true proportion of infected colonies is, however, scarcely likely to be less than the figure obtained from this survey.

Every twentieth name was selected from lists of members of each of the County Beekeepers' Associations for the purposes of the survey. Each of these beekeepers was asked to send a sample of 20–30 bees from one of his colonies, this colony to be chosen at random, by drawing lots. Over 1000 beekeepers were approached, and samples were received from about 500, distributed over the whole of England and Wales, with the exception of Pembrokeshire. The samples were examined and classified as clean or diseased.

In many of the smaller counties the total number of samples was too small for any reliability to be placed on the estimate: a somewhat arbitrary grouping of neighbouring counties had, therefore, to be

employed. The main indications, probably not very precise, are of areas of low percentage infection in Cheshire and the east midlands and of high infection in the south and south-west.

The survey was planned only with the object of discovering any large and clearly defined geographical trends. No such trends were found with any clearness, and it seems probable that the distribution of acarine disease over the country is fairly uniform. There were 20·7 % of diseased samples in the 502 examined, and this is likely to be within 3–4 % of the true value. For comparison, 975 routine examinations of samples voluntarily sent in to Rothamsted Experimental Station between May 1941 and June 1942 gave a figure of 25·9 % infected. Comparison of the distribution of the infected samples sent in voluntarily with those included in the survey, shows almost complete lack of agreement in details. On the whole, the results of the routine examinations may be taken as supporting the view that the incidence of acarine disease does not vary very greatly over the country. Taking into account the likelihood that the survey covered too high a proportion of the better beekeepers, it seems reasonable to conclude that at least one colony in five of all those in England and Wales suffers from acarine infection.

Cases of nosema, a disease caused by the parasitic protozoan, *Nosema apis*, which lives in the mid-gut of the honey-bee and causes serious losses, were also recorded in the survey. Only eight were discovered, indicating an infection of 1·6 %, an approximate figure which is almost certainly very much lower than that obtaining in peacetime when there is large-scale importation of bees from the Continent where nosema is very prevalent. These eight cases were, however, widely distributed. The distribution of seventy-five cases of nosema discovered in England and Wales during 1941–2 supports the view that this complaint is very widespread.

Bee paralysis, a series of different complaints due to different causes but all alike in outward signs, would not appear to any extent at the time of year when the survey was made and so was neglected. *Amoeba* disease, a complaint caused by infestation of the Malpighian tubules of the adult bee by a protozoan, has not been discovered by Rothamsted workers in recent years. It is common on the Continent, and its apparent scarcity in this country at the present time would seem to be a powerful argument for the control of importation when it commences once more.

Brood diseases of the honey-bee

By P. S. Milne, *Rothamsted Experimental Station, Harpenden, Herts*

Of the four brood diseases with which beekeepers in Britain are chiefly concerned one is due to a defective queen, another is caused by a fungus, and the remaining two are bacterial infections. A disease called stone brood, caused by the fungus *Aspergillus flavus* Link, is found in Europe but has not yet been reported here, and cases of the virus disease known as sac-brood are rare.

Addled brood

Addled brood is due to some defect in the queen of the affected colony, but the exact nature of the defect is not known. The brood dies after the cells have been sealed, very often not until the young bees are almost due to emerge. There is no evidence of any bacterial or virus infection, and the dead larvae and pupae usually decompose by autolysis. Requeening with a queen which produces normal brood is all that is necessary for successful control. Diagnosis is complicated by the fact that the appearance of the dead brood is sometimes indistinguishable from that due to accidental causes such as chilling or starvation, and it is probable that many supposed cases of addled brood are actually the result of bad weather conditions or of bad management by the beekeeper.

Chalk brood

Chalk brood takes its name from the chalky white appearance of the dead brood. It is caused by the fungus *Pericystis apis* Maasen. The spores of the fungus germinate either on the surface of the larva or in the gut; the hyphae penetrate the tissues and cause the death of the larva soon after it has been sealed over by the bees, transforming it into a fluffy white or yellowish white 'mummy'. These mummies are often exposed to view as a result of the removal of cell cappings by the bees in their efforts to clear the dead brood out of the comb. They fill the cells completely at first and have a rubber-like consistency; later they shrink and become hard brittle objects, sometimes turning a greenish brown or black colour owing to the formation by the fungus of cysts containing spores. As a rule only small patches of brood are affected and the disease is seldom a serious source of loss. In mild cases the colony will often overcome the attack without treatment; in more severe cases some of the combs may have to be destroyed and the bees transferred to a fresh hive fitted with clean drawn comb or foundation. Strong colonies kept in dry, well-ventilated hives are the best form of defence against the disease.

Foul brood

The term 'foul brood' includes two separate bacterial diseases, one known in the English-speaking world as European foul brood and the other as American foul brood. In the German literature the terms *gutartige Faulbrut* or *Sauerbrut* (E.F.B.) and *bösartige Faulbrut* (A.F.B.) are usual. The first important paper on the subject was published in England by Cheshire & Cheyne in 1885. Cheshire described the pathogenic history of foul brood, and his description, drawn from his entire experience as a beekeeper, covers the symptoms of what are now known to be the two distinct diseases; Cheyne investigated the bacteriology of foul brood, apparently from a single specimen of comb given to him by Cheshire, and described a bacillus isolated from it. Later work by White, in America, and others led to the recognition of two sets of symptoms, each with its own associated bacterial flora, and the foul brood of Cheyne came to be recognized as 'European' foul brood to distinguish it from the 'American' foul brood of White. The names have therefore no real geographical significance and both diseases occur in Europe and America.

American foul brood is caused by the spore-bearing *Bacillus larvae* White. The spores are taken in by the larvae with its food and remain in the gut without germinating until the feeding of the larva ceases and the cell is sealed over by the bees. Germination then takes place, the vegetative cells multiply rapidly and within a short time the larvae collapses and dies. Spores are again formed by the bacillus and these remain dormant until they are ingested by another larva. They are very resistant to exposure, heat and disinfectants and retain their viability for many years in old combs and derelict hives. The bacillus is intolerant of the presence of other bacteria and normally occurs in pure culture in the diseased larva.

The symptoms of the disease are remarkably uniform in all cases. The pearly-white colour of the fully grown larva changes to a creamy brown, the larva loses its shape and becomes a slimy mass lying on the floor of the cell. At this stage the larval remains show a marked ropiness when disturbed with a probe, and this condition constitutes one of the most reliable diagnostic features. There may also be a foul smell from the decomposing larva, which is perhaps best described as being like that of an ill-kept glue-pot. Later the remains dry down to form a hard, almost black scale of a characteristically uniform shape, covering the greater part of the lower angle of the cell and extending right back to the base. Associated with the collapse of the larva within the sealed cell are changes in the appearance of the capping. It becomes moist and dark in colour, and as the larval remains shrink it is drawn into the cell; small holes or cracks appear and eventually it may be chewed away completely by the bees working on the comb. Diseased combs often have a patchy appearance owing to these open cells containing dead larvae or scales being interspersed with normal brood.

Spores from the dead brood are spread throughout the hive by the activities of the workers. Some may be given directly to young larvae along with the brood food, and others get into the honey stored in the brood combs and supers for future use in the preparation of more brood food. Consequently, once the disease is established more and more larvae become infected, the colony becomes progressively weaker in bees and sooner or later it dies out. Such colonies, or those which are already weakened by the disease, may be robbed by bees from strong colonies kept in the neighbourhood and spores are then carried back by the robbers in the stolen honey. Thus it happens that when the disease first appears in an apiary it is often the strongest colony which is found to be affected.

Quite apart from direct robbing of this kind the fact that the honey is contaminated opens up almost unlimited possibilities for the spread of American foul brood. Honey extracted from infected colonies may be exposed to flying bees during the various processes leading up to the bottling and distribution of the finished product. Also the empty supers are usually put back on the hives for the bees to clean out, and it may easily happen that combs from a diseased colony are given to a healthy one. The honey remaining in such combs is taken down by the bees into the brood chamber and the spores in it will find their way into the brood food, either in the autumn or in the following spring. The beekeeper may be responsible for spreading the disease in various other ways. For example, many of the routine manipulations involve the transfer of bees and combs from hive to hive, and in this way an undetected infection can soon be spread throughout the apiary. The buying and selling of bees and second-hand equipment from diseased apiaries also results in the spread of infection from one locality to another, particularly in wartime when high prices are offered in advertisements and at auction sales.

European foul brood is now generally recognized as being caused by a non-spore-forming organism hitherto known as *Bacillus pluton* White. Until recently all attempts to culture it *in vitro* have failed and complete proof that it is the causative agent has been lacking. Various other bacteria invade the diseased larvae, and among the earliest to be found is a rod-like form which was given the name of *Bacterium eurydice* by White in 1912. Since then it has been regarded as of secondary importance only, but now Burri in Switzerland claims that it and *B. pluton* are two forms of a single organism and that both forms will grow on ordinary laboratory media. Confirmation of this claim should lead to a better understanding of the etiology of the disease. Other secondary bacteria appear at a later stage, and the course of the disease is modified according to which of these predominates. Consequently the symptoms

of the disease are rather variable, in contrast to the uniform picture presented by American foul brood.

The young larvae are infected by the ingestion of contaminated brood food and die at about the fourth day from the hatching of the egg. The plump, pearly white appearance is lost, the larva collapses into a shapeless mass, turns yellow or brown, and later dries to form a loosely attached scale. The consistency of the freshly dead larva varies: it may be sticky or granular but never ropy. The smell of the decomposing remains also varies, both in intensity and quality: it may be exceedingly foul or merely sour, depending upon which of the secondary bacteria has become established after the initial infection. Some of the infected larvae may survive until their cells are capped, but the disease is essentially one of the unsealed brood, again in contrast to American foul brood where the symptoms appear only after the larva is fully grown and is already preparing to change to the pupa.

The proportion of dead larvae may be sufficiently high to cause the death of the colony owing to the reduction in the output of worker bees. In other cases there is a marked seasonal periodicity, with a peak in the proportion of diseased larvae in the spring followed by a decline which may lead to a disappearance of all visible signs at the end of the season. A reappearance of the disease is, however, likely in the following spring, especially if the normal expansion of the brood nest at this time is retarded by bad weather. Within the hive the infection is spread by the attentions of the nurse bees to the young brood. Some larvae with a mild infection may pupate and develop into apparently normal workers, but they are unable to spin a proper cocoon and succeed only in smearing their cells with a deposit containing *B. pluton*; in biting their way through the cappings the young bees emerge with their mouth-parts already contaminated, and they act as carriers during the operations of cell-cleaning and the preparation and distribution of brood food. Nectar stored in contaminated cells must be regarded as a source of infection, but as *B. pluton* does not form spores, and is said to be killed by prolonged contact with honey, the latter is probably not so dangerous as that from cases of American foul brood.

Robbing, careless manipulation, the transfer of combs, and particularly the drifting of young bees, many of which may be carriers, all play a part in the spread of the disease from one colony to another.

Occurrence and distribution of foul brood

American foul brood is widely distributed in Britain and in some areas it is very prevalent. Samples of comb submitted to Rothamsted Experimental Station during 1942 show that it is present in most of the counties of England, in parts of Wales and Scotland and in Northern Ireland: notably in the south and south-west (Hampshire, Wiltshire, Dorset, Somerset and Devon), in the south-east (Essex, Middlesex, Surrey and Kent) and in the west midlands (Warwickshire, Worcestershire and Herefordshire). Out of a total of 958 samples received from March to September 555 were found to be infected with American foul brood, many of them advanced cases from which new ones are likely to have arisen. For various reasons it has not been found possible to hold a sampling survey for brood diseases similar to that recently held for adult-bee diseases; the alternative of visiting apiaries and examining colonies on the spot is also not feasible on a large scale at the present time, though a local survey of this kind was carried out during 1941 in the north of Scotland. Here a total of 1233 colonies was examined in 331 apiaries, and American foul brood was discovered in 17 % of the apiaries and 7 % of the colonies.

European foul brood is much less prevalent; in 1942 only fourteen cases were diagnosed at Rothamsted, eight from Hampshire, two from Dorset, and one each from Monmouthshire, Warwickshire, Wiltshire and Northern Ireland. It should be remembered, however, that this disease is more easily overlooked in the hive than American foul brood, and in those cases where it disappears during the course of the summer the beekeeper may not realize what is wrong, even if he has seen some abnormal brood in the spring. In the Scottish survey it was found in two apiaries only.

The control of foul brood

No cure, in the strict sense of the word, is known for either of the two foul brood diseases; no drug has been found which will prevent the death of an infected larva or which will stop the spread of infection within the colony, and no reliable means of disinfecting combs from diseased colonies has been devised.

For American foul brood, the only practicable method of treatment is to attempt to rid the colony of the disease by shaking the bees from the combs and transferring them to a clean hive. Any infected honey which the bees take with them is consumed in the process of comb-building, and when the first eggs hatch the risk of the young larvae becoming infected is diminished. The results are, however, always uncertain, and the operation can only be carried out during a honey-flow when the risk of the exposed combs being robbed is at a minimum. An improvement on this shaking treatment, known as the saltpetre method, has been developed during recent years. The bees are stupefied quickly, before they have time to fill themselves with honey, with the fumes of burning saltpetre; they fall off the combs in a heap on the floor of the hive, and before they revive the rest of the hive is removed and replaced by a clean brood chamber containing frames and foundation. In this way the bees can be saved, but all the honey and combs must be destroyed and the hive parts must be thoroughly scorched with a

blow-lamp before they are used again. The method is justifiable only with strong colonies in which the disease has not made much progress, and when skilled help is available to plan the operation beforehand and to supervise the destruction of the infected material. A supply of sugar for feeding the bees in their new and empty hive is also necessary. If these conditions are not fulfilled the destruction of the whole colony is the only alternative.

Cases of European foul brood cannot be dealt with satisfactorily by the saltpetre method, probably owing to the presence of carriers among the young bees. Even where the outward signs of the disease disappear during the summer months it is not safe to incorporate the colony into the general scheme of apiary management and it must be treated as a separate unit, with its own set of combs and other equipment. In the meantime the infection may already have been carried by drifting nurse bees to neighbouring hives, in which it may remain dormant until the following season. For this reason European foul brood is not easy to eliminate from an apiary, and prompt destruction of each case as soon as the symptoms are recognized is the safest policy in the long run.

Control of foul brood on a national scale has been hampered by the inability of most beekeepers to recognize it when it appears in their colonies, and also by the fact that in some circles the open discussion of foul brood has been regarded as taboo owing to a fear of unfavourable reactions among the honey-consuming public. Honey from infected colonies is perfectly fit for human consumption, but the notion persists that anything which detracts from the conception in the public mind that the honey-bee is a clean and virtuous insect should be avoided. This attitude is being overcome by the efforts of the more enterprising beekeepers' associations and other bodies throughout the country; by means of lectures, photographs and other kinds of propaganda, beekeepers are being given the opportunity of getting to know more about brood diseases and are learning how to recognize them and to seek expert advice whenever signs of trouble are noticed.

In order to encourage and assist the eradication of disease a scheme of insurance against foul brood was started in 1936 under the name of Bee Diseases Insurance, Ltd. Members of those beekeepers' associations which subscribe to the scheme receive compensation for colonies which are treated or destroyed by the local B.D.I. representatives; no claims are considered for colonies which have already died out before the presence of foul brood is reported. Much valuable work has been done during the past 6 years, but it was clear that many cases of foul brood were not being reached by this voluntary co-operative effort and that stronger measures were needed. The outbreak of war in 1939 provided an opportunity for further action, and the Rothamsted Bee Research Advisory Committee, at the invitation of the Ministry of Agriculture and Fisheries, drew up a plan for the control of foul brood in England and Wales by means of legislation. The plan was submitted to beekeepers for their criticisms, and after various modifications it eventually became law as the Foul Brood Disease of Bees Order, 1942. It provides for the inspection of any premises on which bees are kept within a radius of 3 miles of a suspected outbreak of foul brood, and for the compulsory destruction, without compensation, of all infected colonies discovered in that area. The Order is administered by the County War Agricultural Executive Committees, who appoint competent beekeepers to act as local unpaid inspectors when the need arises. Hives containing live bees may not be examined by an inspector during the winter months (1 Oct.–31 Mar.), and both the beekeeper and the inspector are safeguarded against incorrect diagnosis by a clause requiring the submission of a sample comb from each suspected colony to an approved institution, whose report must confirm the presence of disease before the owner can be instructed to destroy his bees. The Order is intended to supplement the activities of beekeepers' associations (many of whom already have their own bee disease services) and of Bee Diseases Insurance, Ltd., who have now made it possible for non-members of beekeepers' associations to avail themselves of the benefits of insurance at a slightly increased premium. It enables action to be taken against foul brood in apiaries whose owners do not co-operate in local efforts to eliminate disease, and whose neglect is endangering the health of other bees kept in the neighbourhood. During the period of April–September 1942, 282 cases of foul brood were dealt with under the Order: 278 of American foul brood, three of European foul brood, and one case of both diseases in the same colony.

The annual losses caused by foul brood in this country are heavy; not only is there the material and financial loss to the beekeeper himself to be considered, but there is also a reduction in the efficiency of the bee population as a whole, both as a pollinator of orchard and field crops and as a direct source of food in the form of honey. In addition, beekeepers are often discouraged by their experiences with disease and much valuable equipment lies idle, or what is worse, is neglected and left as a source of infection to other people's bees. The position can be improved only by spreading a knowledge of the essential facts about foul brood and by the encouragement of better beekeeping in every possible way. The main responsibility for this must rest with the beekeepers' associations, whose co-operation and support is essential if further progress is to be assured.

Orchard spray poisoning of the honey-bee

By G. A. Carter, '*June*', *West Farm Close, Ashstead, Surrey*

The intensification of agriculture has brought to the fore the question of the spray poisoning of the honey-bee. Poisoning of bees may be caused either by collection of the spray itself, or by collection of pollen contaminated by spray. There is no evidence that poisoned nectar is ever collected.

Solutions containing arsenic compounds show no more attraction for the honey-bee than distilled water. Workers at Rothamsted Experimental Station have reported that no cases of poisoning with nicotine, nicotine sulphate, lime sulphur or copper have been obtained either in the laboratory or in cases that have been investigated in the field in this country.

The great majority of bees which have collected arsenic die in the field if the hive is some distance from the orchard concerned. When the hives are very close to the scene of spraying, dead and dying bees are likely to be found around them. Severe weakening of the colony, rather than complete destruction, results. It is suggested that to avoid this loss, winter spraying with petroleum oils during bud dormancy should be efficiently carried out, and that arsenate spraying should only be done in conjunction with lime sulphur, after petal fall. The lime sulphur acts as a repellent. Provision of water within the hive by means of frame feeders is also helpful.

Work on bee repellents. Management of colonies for pollination

By C. G. Butler, *Rothamsted Experimental Station, Harpenden, Herts*

Work on bee repellents

Preliminary investigations have suggested that both lime sulphur and nicotine sulphate are repellent to bees, and that their inclusion in arsenical spray mixtures renders the latter innocuous to the honey-bee provided they are not applied to open flowers. In order to obtain more definite quantitative data, a series of experiments was carried out to determine the degree of attraction or repellence to honey-bees of each of the ingredients in the more usual spray mixtures. The technique employed was to offer a given number of bees a powerful attractant, sucrose, and to measure the degree of repellence from it caused by the addition of the various ingredients used in spray mixtures.

It was found that lime sulphur, nicotine and nicotine sulphate, in the concentrations used in spray mixtures, are all strongly repellent to honey-bees, both in laboratory and orchard experiments. There is little doubt that the inclusion of one or more of these substances in orchard sprays will prevent the poisoning of honey-bees provided the sprays are not applied to open flowers. This conclusion is supported by data obtained in cases where poisoning has occurred; in every such case poisoning was found to be due to spraying of open blossom, usually with an arsenical spray alone. The deliberate spraying of open blossom with sprays containing arsenic should, therefore, be avoided, and all possible steps be taken to prevent such sprays falling or drifting on to the open flowers of weeds and other plants. This is a counsel of perfection, but in his own interests alone the fruit-grower will be well advised to attempt to follow this rule, since recent work at Rothamsted has shown that a honey-bee foraging on an average flying day spends about four-fifths of its time upon a crop and only one-fifth in the hive; thus the application of an arsenical spray to flowers which bees are working is likely to kill 90% of the honey-bees pollinating these flowers.

* An account of the technique, apparatus employed and results obtained in these trials has already been given (Butler, C. G., Finney, D. J. & Schiele, P., Experiments on the poisoning of honeybees by insecticidal and fungicidal sprays used in orchards, *Ann. app. Biol.* **30**, 143).

Management of colonies for pollination

This subject can conveniently be considered under two headings. First, the preparation of the colonies —a matter that concerns the beekeeper; and, second, the placing and arrangement of colonies within or near a crop—a matter primarily for the grower.

Before more than purely tentative suggestions can be made on the placing of colonies by a grower, it is essential to know as much as possible about the foraging activities of honey-bees—their behaviour when seeking nectar and pollen in the field. This involves close study of the behaviour of individual bees, for which a marking technique has to be employed. In order to simplify matters it is also best

to use an artificial crop, in which the volume and concentration of the nectar, sugar syrup, can be controlled. For this purpose, an experimental field was designed* containing 112 dishes of syrup, the dishes being placed on the arcs of circles, each arc being 20 yd. from any other arc, and each dish on an arc being 20 yd. from each of its neighbours. The nearest dishes were 160 yd. from an apiary and the farthest 400 yd. away. Once the bees were working this artificial crop, various problems could be solved by making counts and noting the movements of marked bees.

Contrary to suggestions that have been made from time to time, the experiments showed that, other things being equal, and on any given crop, the greatest number of foragers are found nearest to the hive. As one moves away from the hive the number of foragers progressively decreases. It was also demonstrated that bees will seldom work flowers growing in even partial shade, although these may contain abundant nectar of high concentration.

It was found that an individual bee becomes very constant in its attention to any one source of nectar or syrup which does not fail, flying in a more or less straight line between it and the hive with great regularity, thus confirming the observations of Minderhoud. The dishes used in these experiments probably represented more or less optimal conditions with respect to nectar secretion. Nevertheless, this same constancy of bees to a small group of flowers, sometimes even to an individual inflorescence, in a crop can be demonstrated. The foraging bee does not wander at random over a crop which is rich in nectar but works only in a small area, usually not more than 4–5 yd. in diameter. Fortunately, however, from the grower's point of view not all the bees in a large population are constant to a particular patch of flowers. Superimposed upon the stable population is a fluctuating population of wandering bees which is responsible for effecting cross-pollination in orchards. This new factor of apparent inconstancy has been demonstrated on several different crops and found to be due, first, to competition which results in a shortage of nectar and a percentage of wandering or drift from one area to another and, secondly, to the advent of young bees which have not yet formed definite habits. It therefore follows that in order to obtain successful cross-pollination in an orchard, a very powerful force of field bees is necessary, resulting in competition and a rise in the percentage of wandering bees of no fixed location. Lack of success where bees have been introduced into a properly arranged orchard is probably due to the use of an insufficient force of bees. It is also necessary that there should be as little competition as possible between the crop requiring pollination and other flowers. Other things being equal the honey-bee will work the crop in which the nectar concentration is highest. This fact often accounts for poor results in plum orchards where many dandelions are growing, the bees working the latter in preference to the plum blossom.

Until it is known how many colonies of bees are required to produce a maximum crop in a particular orchard, it will probably pay to place the colonies in a group in the centre of the orchard (two or more groups in irregularly shaped orchards). If the field force of bees is sufficient this should result in an equal set of fruit throughout the orchard. If, on the other hand, the field force is insufficient, the highest yield will be obtained near the hives and the yield of fruit will fall off progressively the farther one moves away from them, until one gets outside the area of competition when the degree of set will remain more or less constant. Careful crop records taken over a number of years should enable the average number of colonies for maximum production to be found.

With respect to other seed crops requiring pollination the position is somewhat different, as the aim is not only to pollinate the crop but also to avoid cross-pollination from other plants of the same or closely allied species from outside.

It is obvious that, in order to obtain pure seed, the plots of different varieties must be fairly widely separated. In many districts, however, growers can profitably use honey-bees as pollinators of pure seed crops, though too many colonies, which would only result in excessive wandering, must be avoided. The problem is almost the reverse of that of the fruit-grower, but the number of colonies required can similarly be determined. The cutting of neighbouring flowering crops prior to cutting of the seed crop must be avoided, or the bees working the former will be driven to seek new forage and may readily cause unwelcome competition resulting in excessive wandering on the seed crop.

If bees are placed near a plot A of a variety, there is more chance of wanderers carrying pollen to plot B, farther away, than vice versa. Large areas of a crop will probably show more extraneous mixing around their edges than in the centre, and in such a homogeneous plot it would prove highly satisfactory to place the bees in the centre of the area. Where, on the other hand, there are a number of plots of different varieties of the same species close together, the best place for the bees is almost certainly 200 yd. away in order to avoid the results of excessive wandering.

The problems involved are by no means insuperable and experience has shown that when honey-bees are used intelligently greatly increased yields are likely to be obtained.

* Investigations by C. G. Butler, E. P. Jeffree & H. Kalmus.

The design and interpretation of bee experiments

By D. J. Finney, *Rothamsted Experimental Station, Harpenden, Herts*

Types of experimental design developed primarily for use in agricultural field trials have been adopted in a series of laboratory trials of the repellent effect of certain substances on the honey-bee. An account of the results of these experiments has already been given (Butler, C. G., Finney, D. J. & Schiele, P., Experiments on the poisoning of honeybees by insecticidal and fungicidal sprays used in orchards, *Ann. appl. Biol.* **30**, 143).

Further examination of the relative quantities of sucrose solutions containing different concentrations of lime sulphur or nicotine sulphate taken by bees shows the sigmoid nature of the relationship between log-concentration and uptake. The fitting of the appropriate sigmoid curve to the data is facilitated by an adaptation of the probit transformation, commonly used in toxicological studies. In the two relevant trials, the uptake of nicotine sulphate was, for a given concentration, much less than that of lime sulphur, and the rate of change of uptake with changing concentration was also less for nicotine sulphate, this latter fact suggesting that individual bees show greater variation in their tolerance for nicotine sulphate than for lime sulphur.

The discussion

Compiled by E. P. Jeffree, *Rothamsted Experimental Station, Harpenden, Herts*

In a short discussion, Mr M. H. Moore said that in experiments at East Malling Research Station on the control of scab, 1 % lime sulphur (as well as other sprays) had been applied to fully open apple blossom, and no reduction in yield through lack of pollination following spraying had been found. During the day of spraying the bees did not visit the flowers sprayed with lime sulphur while the latter were still wet (though they did fly about near the flowers, and settled from time to time on the leaves, even drinking the spray fluid there). On subsequent days, however, the bees visited the flowers in apparently normal fashion, and were not deterred by the spray.

These results appear at first sight to be in sharp contradiction to those obtained at Rothamsted Experimental Station (cf. also Shaw, F. R. & Bourne, A. E., *J. econ. Ent.* (1942), **35**, 607), where spraying with 1 % lime sulphur deterred the bees to a considerable degree for several days.

As pointed out by C. G. Butler, recent work at Rothamsted has confirmed that there is soon formed, on a patch of flowers, an 'established population' of bees which is very loth indeed to leave the site and will maintain a memory for it and remain about it even under adverse conditions. They can be regarded as bees upon whom there is a strong *force of attraction* to the particular closely localized site. Foraging bees all eventually become 'established' somewhere or other, but there are also always some 'unestablished' bees (new foragers, as well as bees more thoroughly disturbed from a site of previous establishment). Further, these territories of establishment have been shown to be quite small areas, of the order of 3–4 yd. in diameter only. In respect of these points, the conditions at East Malling and those of the Rothamsted repellent experiments on open apple flowers differed radically. The flowers at East Malling had no doubt reached the peak of the flowering period before spray was applied, and therefore *already* had practically the full number of their established population. At Rothamsted, however, spraying was carried out soon after the first flowers had opened, when the number of 'established' bees was almost certainly very much lower.

Consequently, at East Malling the hungry population of bees belonging to the trees in question would have already a well-formed determination to visit those particular flowers, and to return to them as soon as the lime-sulphur odour had abated enough to allow them to do so. At Rothamsted most of the bees eventually coming to the plots had never worked apple at all before, had no established 'force of attraction' towards any patch, and when they came to find a site to forage avoided those areas that were in the least tainted with the spray.

Further, at Rothamsted the areas sprayed (65 trusses) were definitely much smaller than each individual bee's territory, so that every bee, even if 'established', had an alternative clean area of flowers to go to, within its own territory, and known to it just as well as the sprayed trusses were. If, as is probable, at East Malling the sprayed areas were larger than the territories, the bees (having a 'force of attraction' towards the established territory) had practically no alternative choice but to fly about until it became tolerable for them to re-enter the flowers—or rather, it is probable, until the lime-sulphur odour had abated to just that threshold where the apple scent could at all be detected.

Report of the Council of the Association of Applied Biologists for the year 1942

The Officers and Council of the Association were as follows:

President: H. Martin, D.Sc.

Vice-Presidents: Miss W. E. Brenchley, D.Sc., G. E. Blackman, M.A.

Hon. Treasurer: W. C. Moore, M.A.

Hon. Secretaries: General and Botanical: W. P. K. Findlay, D.Sc.; Zoological: H. F. Barnes, M.A., Ph.D.

Hon. Editors of Annals of Applied Biology: General and Botanical: Prof. W. B. Brierley, D.Sc.; Zoological: C. T. Gimingham, B.Sc., F.I.C.

Council: G. C. Ainsworth, Ph.D., G. E. Blackman, M.A., Miss W. E. Brenchley, D.Sc., D. E. Green, M.Sc., W. E. H. Hodson, D.I.C., *S. E. Jacobs, Ph.D., O. B. Lean, B.Sc., E. A. Parkin, Ph.D., C. Potter, B.Sc., *W. Steer, B.A., *I. Thomas, Ph.D., *W. A. R. Dillon Weston, M.A., Ph.D.

The Council is again able to report a successful year of progress for the Association. The attendance at meetings was on average 50 Members and 30 visitors, which is well above the pre-war figure. The increasing number of visitors at meetings is gratifying, since many of these subsequently become Members and their presence indicates the increasing scope of the Association's work. The total Membership now stands at 329 of whom 34 are known to be resident abroad. During 1942, 24 new Members were elected whilst only 4 resigned. The Council has to record with regret the deaths of E. J. Wortley, E. Walker and W. R. S. Ladell. It is encouraging to note that the number of new Members who have joined since the war is considerably greater than in any previous 3-year period. It is evident that the importance of applied biology in the national economy and in the prosecution of the war effort is recognized much more widely now than it was during the last war, when the Association became more or less dormant.

The following papers were presented to the Association during 1942:

20 Feb. *Symposium on domestic entomology.*†

P. A. Buxton: 'Recent ecological work on the bed-bug.' J. R. Busvine: 'New methods of controlling head and body lice (with film).' K. Mellanby: 'Experimental work on the transmission and development of scabies.' C. G. Johnson: 'Some quantitative aspects of scabies parasitology.' H. G. H. Kearns: 'The control of flies in town and country.' R. C. Fisher: 'Furniture beetles and other wood borers.' G. Fox Wilson: 'Invasion of houses by ants and earwigs.'

17 Apr. Morning session: *Symposium on the pathology of the hop.*

W. M. Ware: 'Hop downy mildew.' W. G. Keyworth: '*Verticillium* wilt and virus diseases.' A. M. Massee: 'Some important pests of the hop.'

Afternoon session: *Discussion on the interpretation of toxicity data.*

H. Martin: 'The significance of the bio-assay in studies of fungicidal action.' A. F. Parker-Rhodes: 'An improved method for the statistical analysis of fungicidal data.' C. Potter: 'Introduction on technique.' D. J. Finney: 'Examples of the planning and interpretation of toxicity tests.'

19 Oct. Joint meeting with the British Mycological Society: *Symposium on potato virus diseases.*‡

G. Samuel: 'Introduction: practical aspects.' F. C. Bawden: 'Some properties of the viruses.' Kenneth M. Smith: 'Some practical difficulties in the production of virus-free potato seed in England.' T. Whitehead: 'Some factors affecting the health of seed potato stocks in North Wales.' I. Thomas: 'Ecology of potato aphides in North Wales.' J. P. Doncaster: 'Potato aphides in Eastern England.' P. H. Gregory: 'The increase of virus disease in the field.' G. Cockerham: 'Potato breeding for virus resistance.'

4 Dec. *Symposium on the honey-bee.*§

C. G. Butler: 'The position of the honey-bee in the national economy. Adult bee disease.' P. S. Milne: 'Brood diseases of the honey-bee.' G. A. Carter: 'Orchard spray poisoning.' C. G. Butler: 'Work on repellents. Management of colonies for pollination.' D. J. Finney: 'The design of bee ex-

* New Members for 1942.

† See *Nature, Lond.* (1942), **149**, 295–6.

‡ See *Nature, Lond.* (1942), **150**, 476–7.

§ See *Nature, Lond.* (1942), No. 3817, **150**, 759–60.

periments.' A. C. FABERGÉ: 'A bee counter for use in studies on bee activity.'

The Council is glad to take this opportunity of expressing its sincere thanks to the authorities of the London School of Hygiene and Tropical Medicine for so kindly affording the Association accommodation for its meetings.

Following on suggestions made at the meeting on 20 Feb., a subcommittee was set up to report on the position of Advisory Entomologists in relation to the control of domestic insects. A copy of a memorandum from this subcommittee has been submitted to the Biology War Committee, who are taking action in this matter. It may be noted that the Biology War Committee, which was set up in 1941, at the request of this Association, in collaboration with two other biological societies, continued to function actively during 1942, and has dealt successfully with a number of the problems which have been submitted to it.

The joint committee set up with representatives from the British Mycological Society to develop a system for the official recognition of pest control products has been dissolved, following the announcement by the Ministry of Agriculture and Fisheries that a scheme for this purpose would shortly be initiated.

The *Annals of Applied Biology* was maintained during 1942, but publication of the issues was slightly delayed owing to production difficulties. Towards the end of 1942 notice was received of a further cut in the paper quota for all journals, and it was decided to meet the need for drastic economy in paper by producing the *Annals* in a 2-column format with a smaller fount, by which means the amount of textual matter per page could be increased. Before submitting papers for editorial consideration Members should make sure that their tables and text-figures are in accordance with the new format. It remains imperative that authors should condense their typescripts to the utmost, delete every non-essential word, table and illustration, and reduce proof corrections to a minimum.

Vol. **29** (1942) comprised pp. ix + 439 and 15 plates as against pp. ix + 416 and 14 plates for vol. **28**. Including papers published in 'Proceedings', vol. **29** comprised 61 papers and 4 reviews, 48 of the former being by Members of the Association. The papers may be classified roughly as follows: general applied botany, 10; mycology and fungus diseases, 5; bacteria and bacterial diseases, 2; viruses and virus diseases, 6; entomology and insect pests; 19; plant protection, insecticides and fungicides, 13; helminthology and nematode diseases, 1; applied microbiology, 1; general, technique and statistics, 4.

The several issues of the *Annals of Applied Biology* were published on the following dates in 1942: No. 1, 6 March; No. 2, 25 June; No. 3, 16 September; No. 4, 24 December.

Report of the Honorary Treasurer for the year ended 31 December 1942

During the year ended 31 December 1942, the amount received for subscriptions and entrance fees, including arrears, was £376. 4*s*. 6*d*., which was £32. 14*s*. 0*d*. more than the previous year. Subscriptions not paid by the end of the year, but considered good, amounted to about £15. One Life Membership compounded subscription of £25 was received and this has been placed in a special reserve fund.

Income from the sale of the current volume of the *Annals of Applied Biology* amounted to £493. 6*s*. 10*d*., a decrease of £27. 16*s*. 10*d*., and there was also a decrease of just over £11 in the sales of reprints. The volume contained 439 pages compared with 416 pages in 1941, and the cost of producing it rose to £949. 16*s*. 6*d*., an increase of £139. 16*s*. 6*d*., due chiefly to an increase of 20% in printing costs. The balance against the *Annals* on the year's working was appreciably offset by an unexpected increase of £67. 9*s*. 0*d*. in the amount received from the sales of back volumes.

The income for the year exceeded the expenditure by £11. 15*s*. 6*d*., and after all obligations have been met, the assets of the Association amount to £1000. 4*s*. 9*d*., of which £694. 16*s*. 3*d*. is represented by National Savings Certificates, and £195. 3*s*. 0*d*. by the estimated value of the stocks of the *Annals*. The total also includes a sum of £60 allocated to the Association by the Council of the Royal Society from the special Rockefeller Foundation Grant in aid of Scientific Publications, a gift I acknowledge with gratitude on behalf of the Association.

W. C. MOORE
Hon. Treasurer

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1942 *Cr.*

Expenditure	£ s. d.	Income	£ s. d.
To Estimated Value of Stock, 1 January 1942	200 17 0	By Sales—Current Volume	493 6 10
To Cambridge University Press	949 16 6	By Sales—Back Volumes, Parts and Sets	82 1 2
		By Sales of Reprints	47 3 8
		By Contribution towards cost of Papers	16 6
		By Estimated Value of Stock, 31 December 1942	195 3 0
		By Balance, carried down	332 2 4
	£1150 13 6		£1150 13 6

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1942 *Cr.*

Expenditure	£ s. d.	Income	£ s. d.	£ s. d.
To *Annals of Applied Biology* balance brought down	332 2 4	By Members' Subscriptions:		
To Printing and Stationery	3 5 7	Arrears	19 5 6	
To Postages and Cheque Stamps	11 14 5	Entrance Fees	9 9 0	
To Subscription—Parliamentary Science Committee	5 5 0	Current	347 10 0	
To Grant—Biology War Committee	8 2 4			376 4 6
To Sundry Out-of-Pocket Expenses of Editors, Secretaries and Treasurer	15 17 0	By Interest on National Savings Certificates		15 1 0
To Audit Fee	4 4 0	By Interest on Bank Deposit Account		1 0 8
To Balance, being Excess of Income over Expenditure for the year	11 15 6			
	£392 6 2			£392 6 2

BALANCE SHEET, 31 DECEMBER 1942

Liabilities and Surplus	£ s. d.	£ s. d.	Assets	£ s. d.	£ s. d.
Sundry Creditors:			Cash at Bank:		
Cambridge University Press	352 4 10		Current Account	245 12 8	
Audit Fee Reserve	4 4 0		Deposit Account	276 0 0	
Sundry Expenses	11 19 4				521 12 8
		368 8 2	500 National Savings Certificates		694 16 3
Subscriptions and Entrance Fees, paid in advance		17 19 0	Stock of *Annals of Applied Biology* at estimated value		195 3 0
Life Membership Fund Reserve		25 0 0			
Excess of Assets over Liabilities:					
As Balance Sheet of 31 December 1941	928 9 3				
Add: Balance of Income and Expenditure Account for 1942	11 15 6				
Rockefeller Foundation Grant (per Royal Society)	60 0 0				
		1000 4 9			
		£1411 11 11			£1411 11 11

W. C. MOORE, *Hon. Treasurer*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, voucher sand documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association according to such books, vouchers and documents.

(Signed)
H. J. COX & CO.
Incorporated Accountants
Auditors

HARPENDEN, 5 *February* 1943